

Figure 1

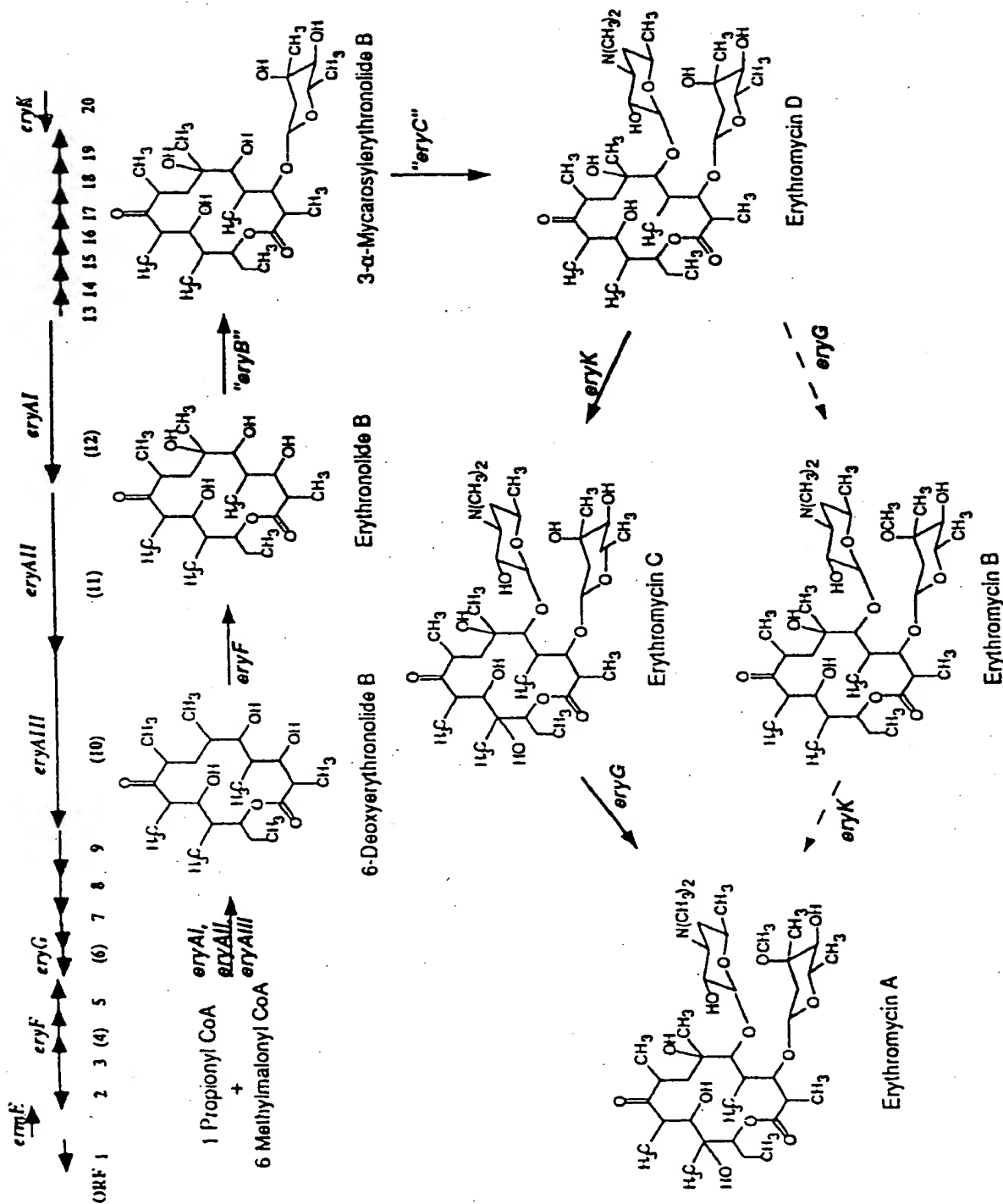


Figure 2

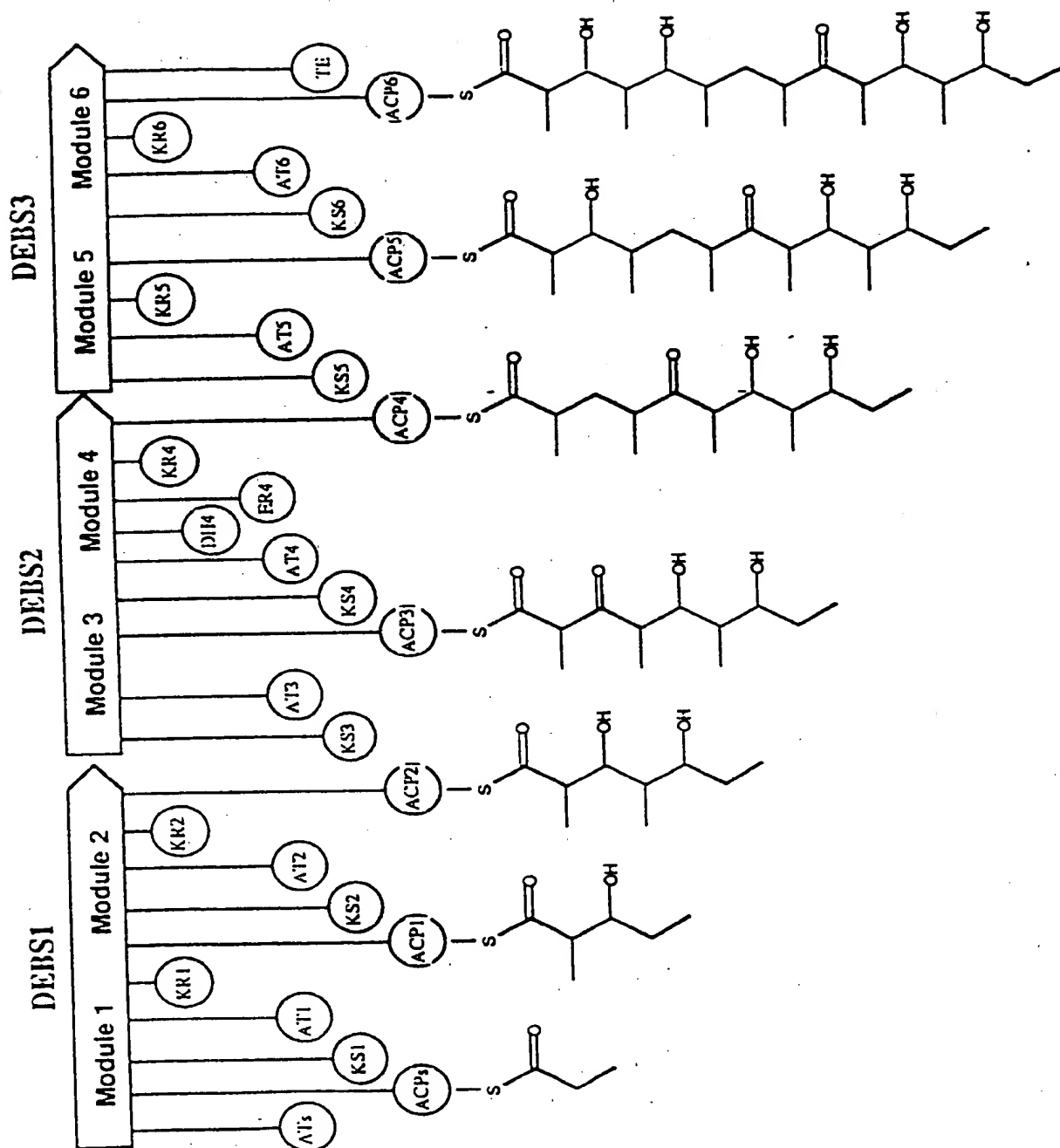
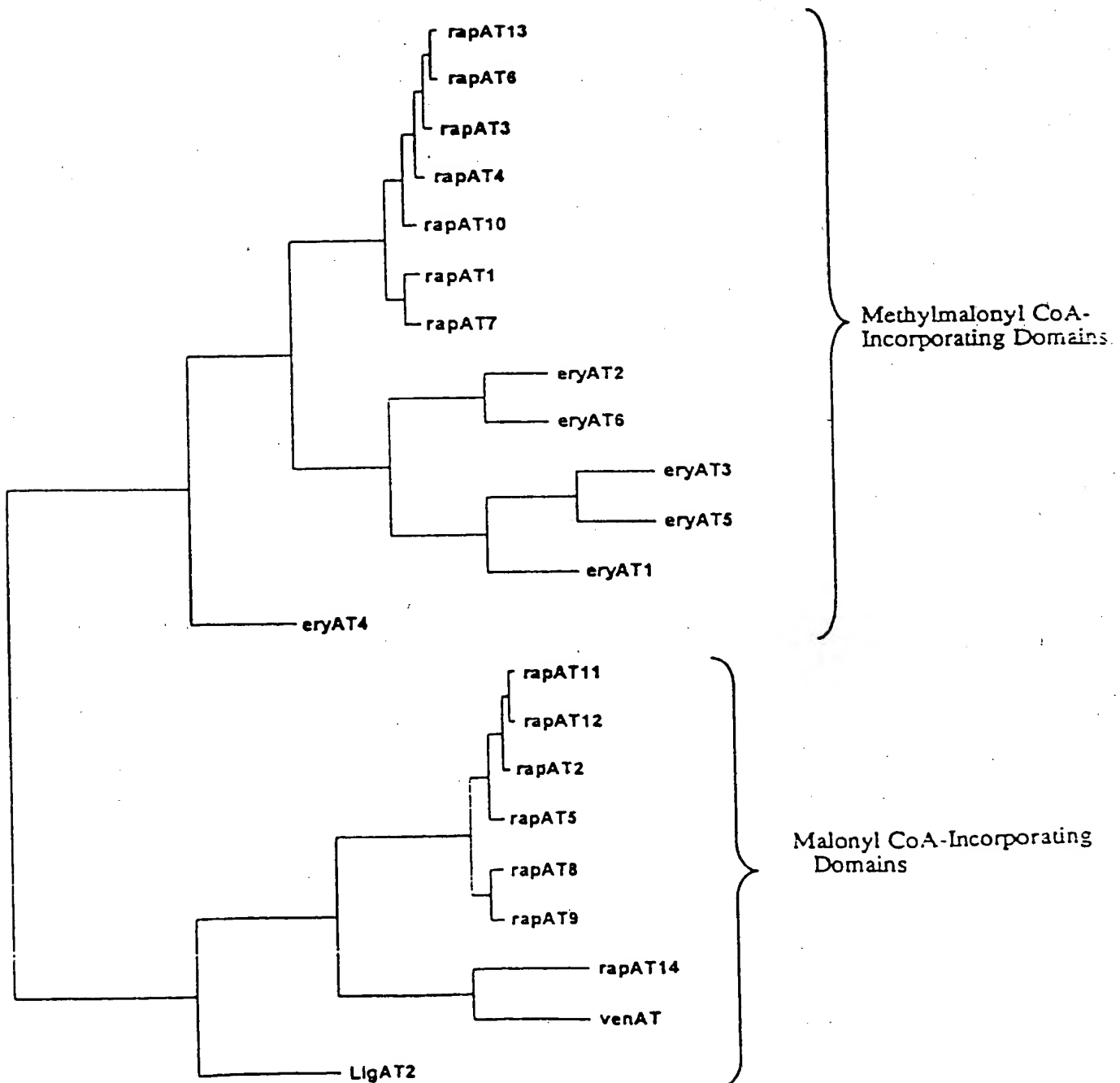
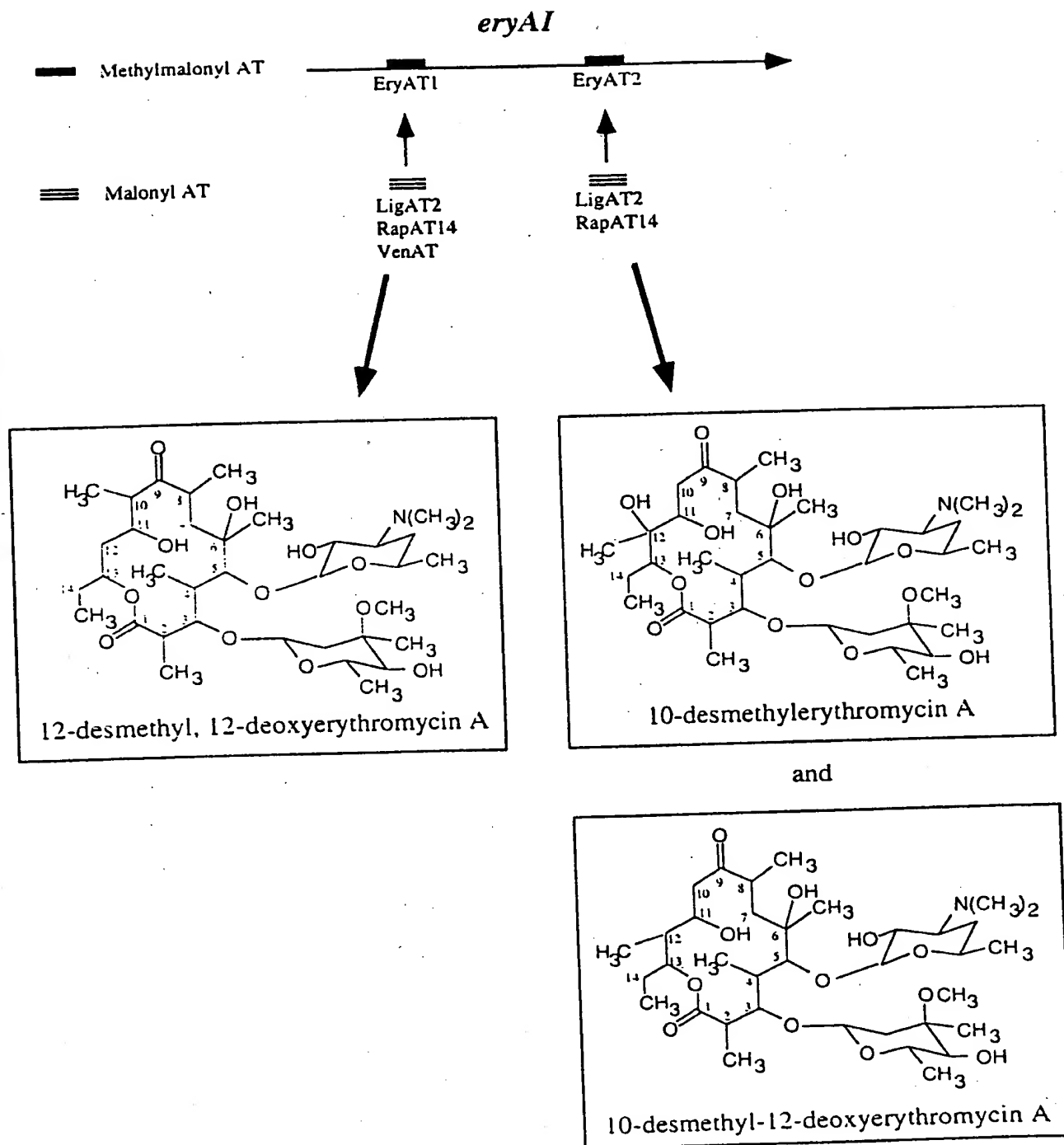


Figure 3



09735056-121100

Figure 4a



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Figure 4b

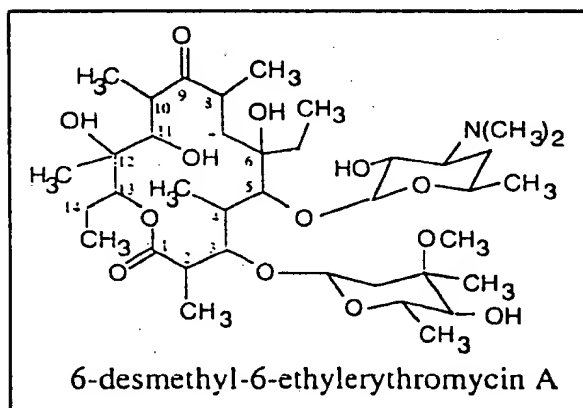
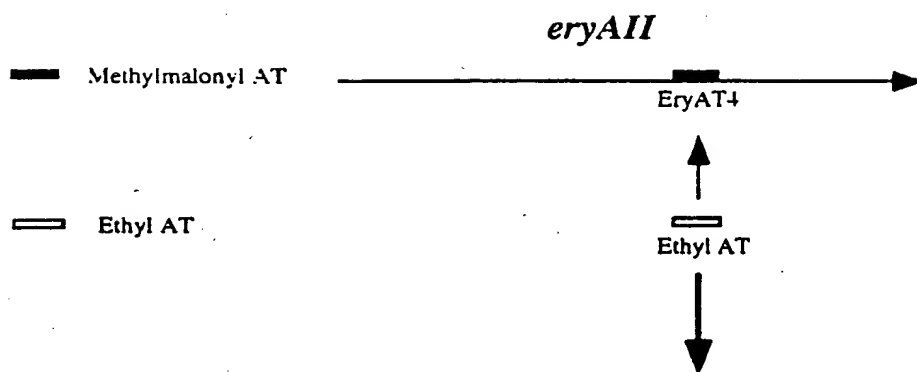


Figure 5

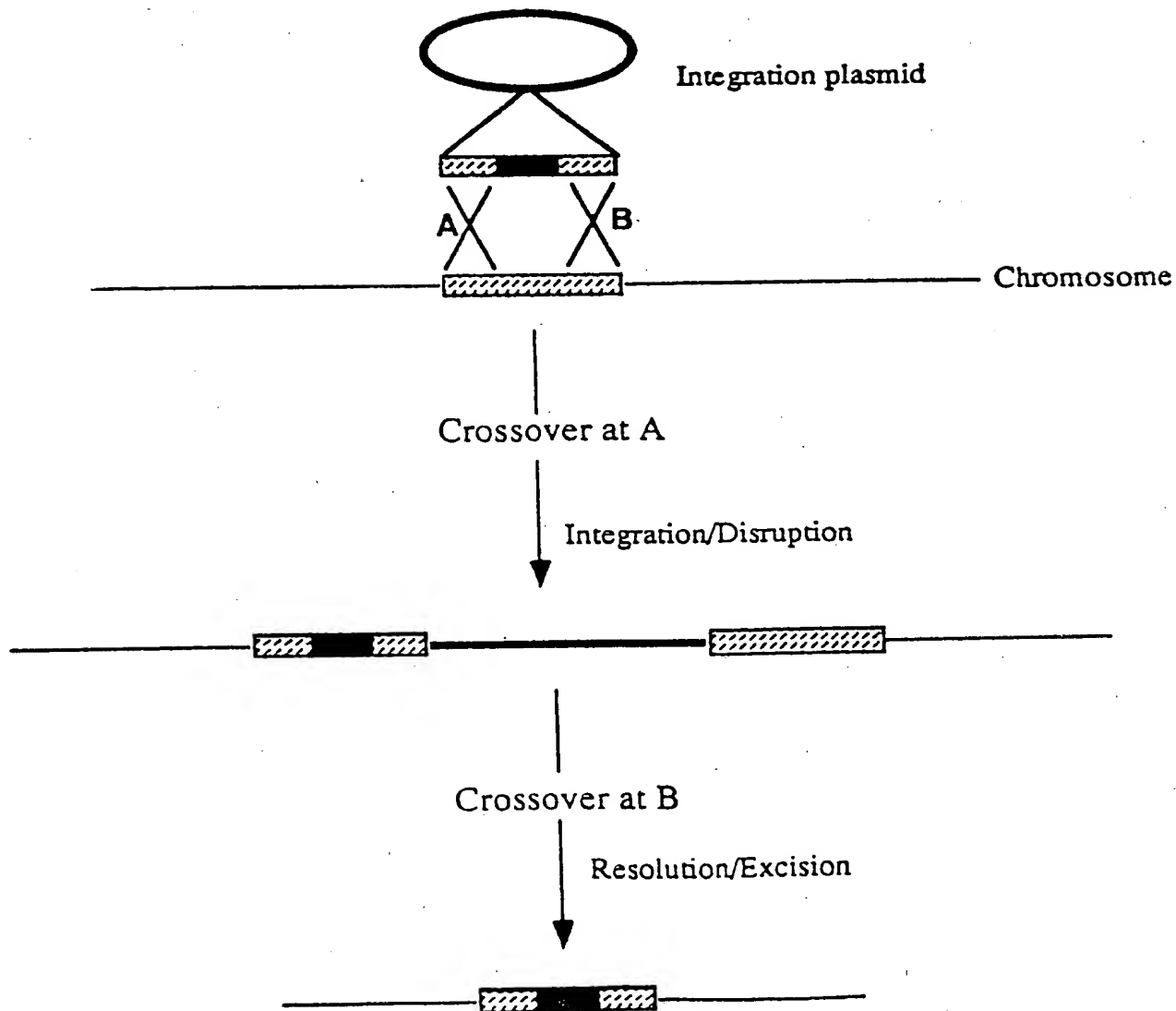


Figure 6

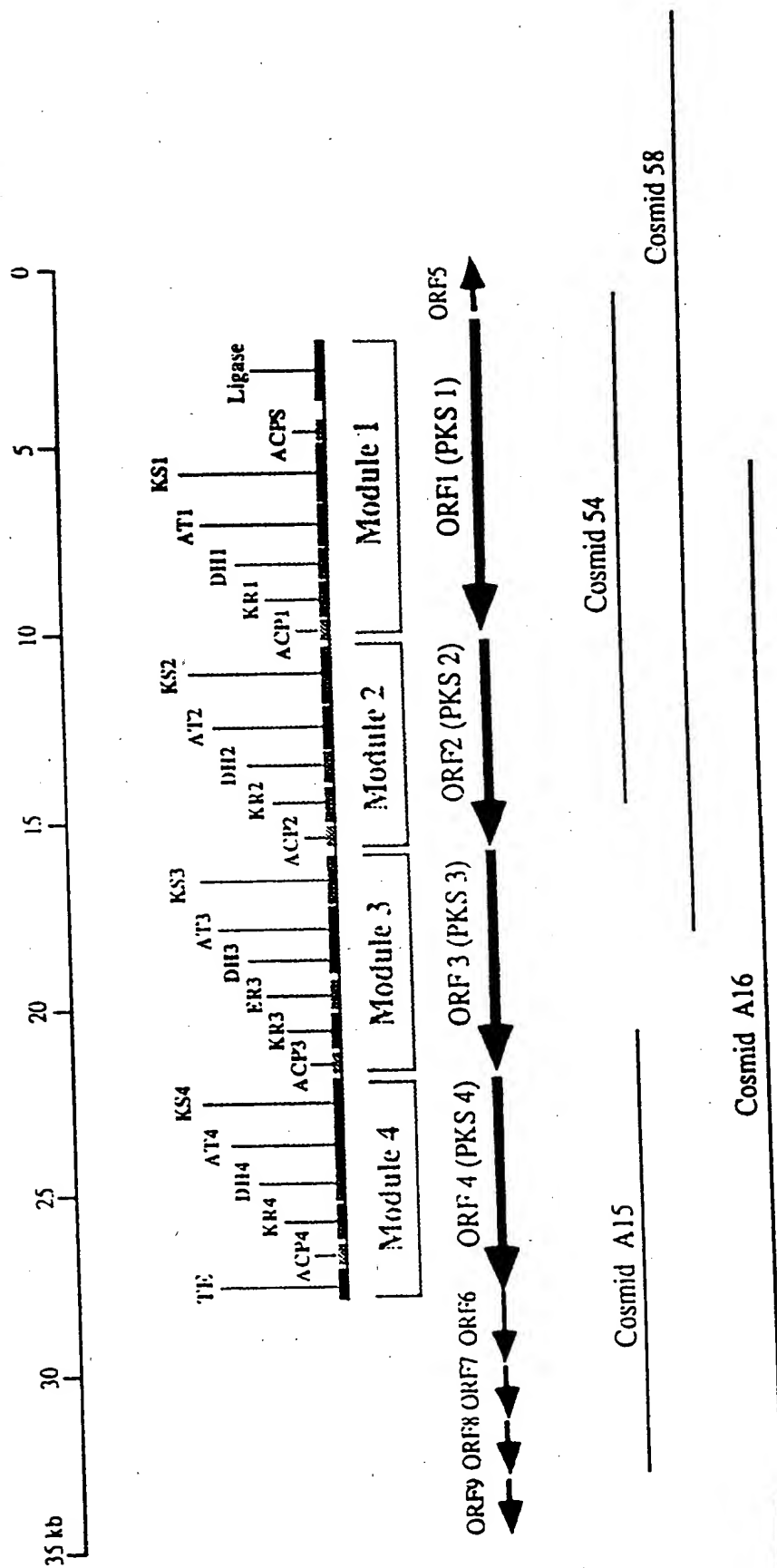


Figure 7

GGGCCGCTGGCGGTGATGTTACCGGACAGGGCTCCCAACGCCCCGGCATGGGACGACAG 60
 G P L A V M F T G Q G S Q R P G M G R Q 20
 TTGTACGAGCACTTCCCCGTCTTCGCCCAGGCACTGGACGAGGTCTTCGCACTCGCCACC 120
 L Y E H F P V F A Q A L D E V F A L A T 40
 CCGGACTACGCGAGGTGATGTTGACCCCGACCAGGCCGAAACACTCCAACGCACCGAC 180
 P G L R E V M F D P D Q A E T L Q R T D 60
 CACGCCCAGATCGCCCTGTTTCGCCTTCGAAACCGCCCTCTACCGACTCTGGGAATCCTGG 240
 H A Q I A L F A F E T A L Y R L W E S W 80
 GGCCTGCGACCCGACATGGTCTGCGGACACTCGGTGCGAGAAATCACCGCAGCCACGTC 300
 G L R P D M V C G H S V G E I T A A H V 100
 TCCGGCACCCCTCACCCCTCCCCGACGCCGTCCACCTCGTCACCACACGCGGCACCCCTCATG 360
 S G T L T L P D A V H L V T T R G T L M 120
 CAAAACCTGCCCCCGGGCGGCCATGCTCGCCGTGCGCCACCGACCCCCACACCCCTCCAA 420
 Q N L P P G G A M L A V A T D P H T L Q 140
 CCCCACCTCGACAACCACGACACCATCTCCATCGCCGCCATCAACGGCCCCCAGGCC 480
 P H L D N H H D T I S I A A I N G P H A 160
 ACCGTCCCTCTCCGGCGACCGCACCACCCCTCCACCACATCGCCACCCAACTCAACACCAAA 540
 T V L S G D R T T L H H I A T Q L N T K 180
 ACCAACTGGCTCAACGTCAGCCACGCCTTCCACTCCCCCCTCATGCAACCCATCCTCCAA 600
 T N W L N V S H A F H S P L M Q P I L Q 200
 CCCTTCACCACCACCCTCAACACCCTCACCCACCACCCCCACACACACCCCTCATCAGC 660
 P F T T T L N T L T H H P P H T P L I S 220
 ATGCTCACCGCCACACCCACCCACCCCGACACCACCCACTGGACCCAGCACATCACCGCA 720
 M L T A T P T H P D T T H W T Q H I T A 240
 CCGTCCGCTACACCGACACCCCTCCACCACCTCCACCACCACGGCATCACCACTACCTC 780
 P V R Y T D T L H H L H H H G I T T Y L 260
 GAAATCGGCCCCGACACCACCCTCACCGCCCTCGCCCGCACCACCCTCCCCACCACCACC 840
 E I G P D T T L T A L A R T T L P T T T 280
 CACCTCATCCCCACCACCCGCGCAACCACAACGAAGTCCGCAGCACGAACGAGGCGTTG 900
 H L I P T T R R N H N E V R S T N E A L 300
 GGCAGGGTGTTCAGCGTGGGCCACTCGGTGGACTGGCGGGCCCTCACTCCGACCGGGAGG 960
 G R V F S V G H S V D W R A L T P T G R 320
 CGTACCTCCCTGCCGACGTACCCCT 935
 R T S L P T Y P 328

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Figure 8

PCR oligos:

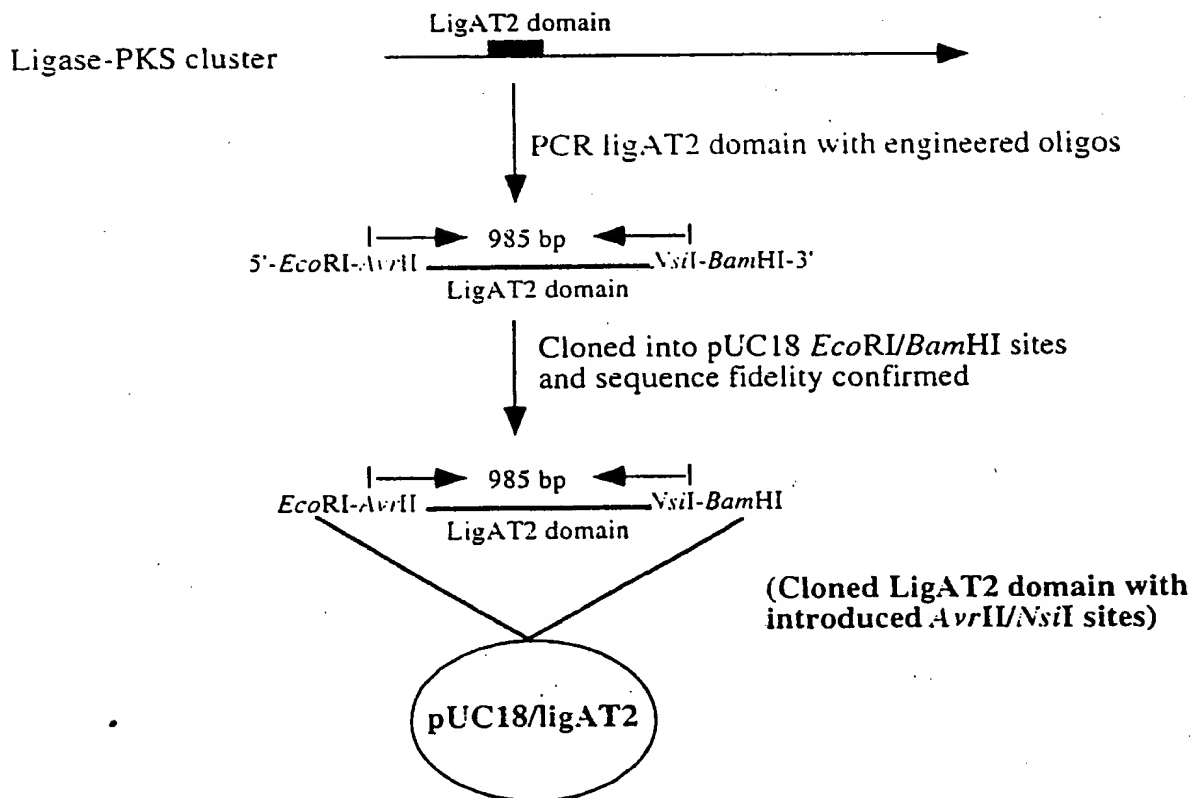
N-terminal Oligo: 5' *EcoRI* Tag-^{AvrII}CCTAGGCTGGCGGTGATGTTCA-3'
GGGCC

Engineered *AvrII* | Homologous region

C-terminal Oligo: 5' *BamHI* Tag-^{NsiI}ATGCATACGTCGGCAGGGAGGTAC-3'
G GG

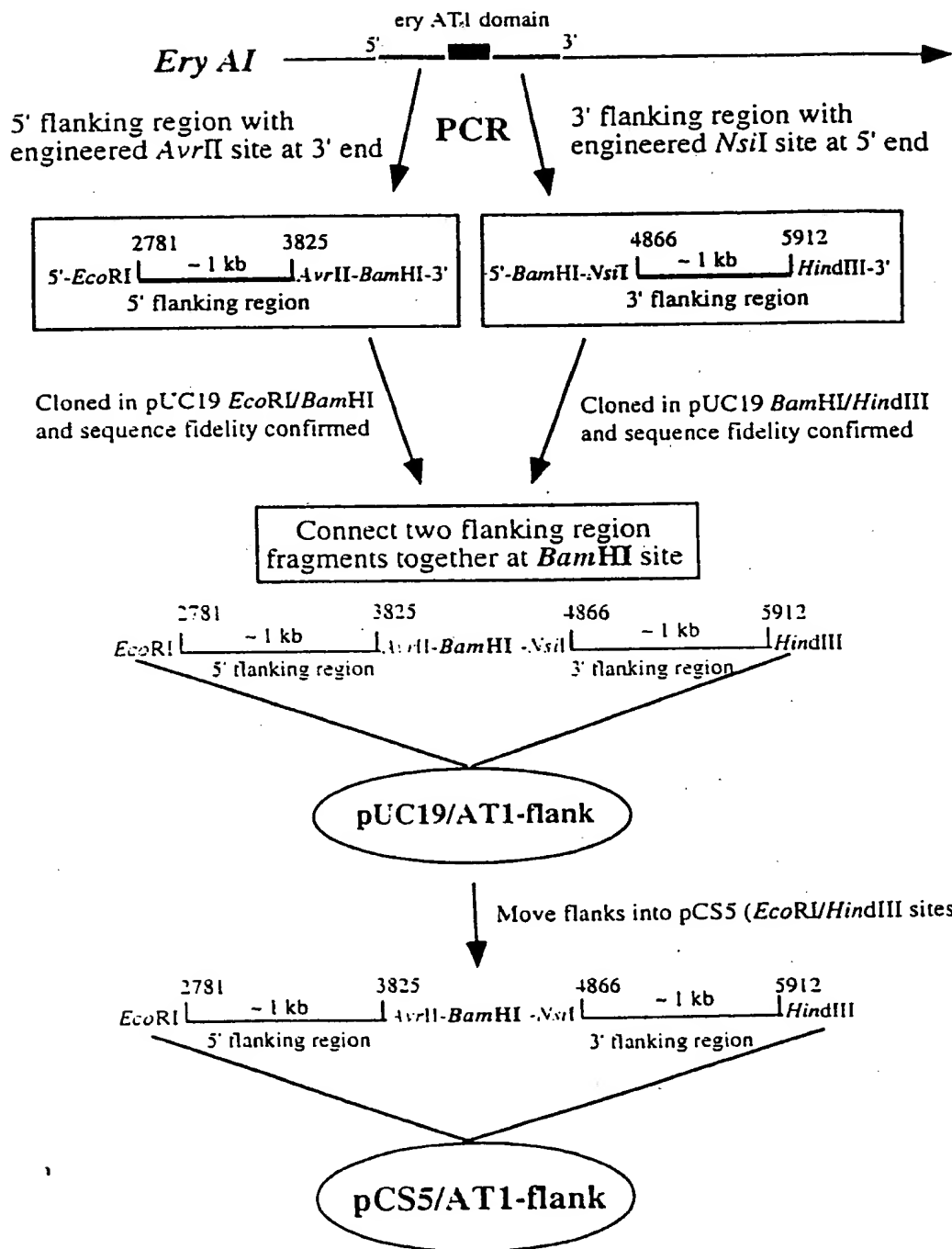
Engineered *NsiI* | Homologous region

PCR cloning:



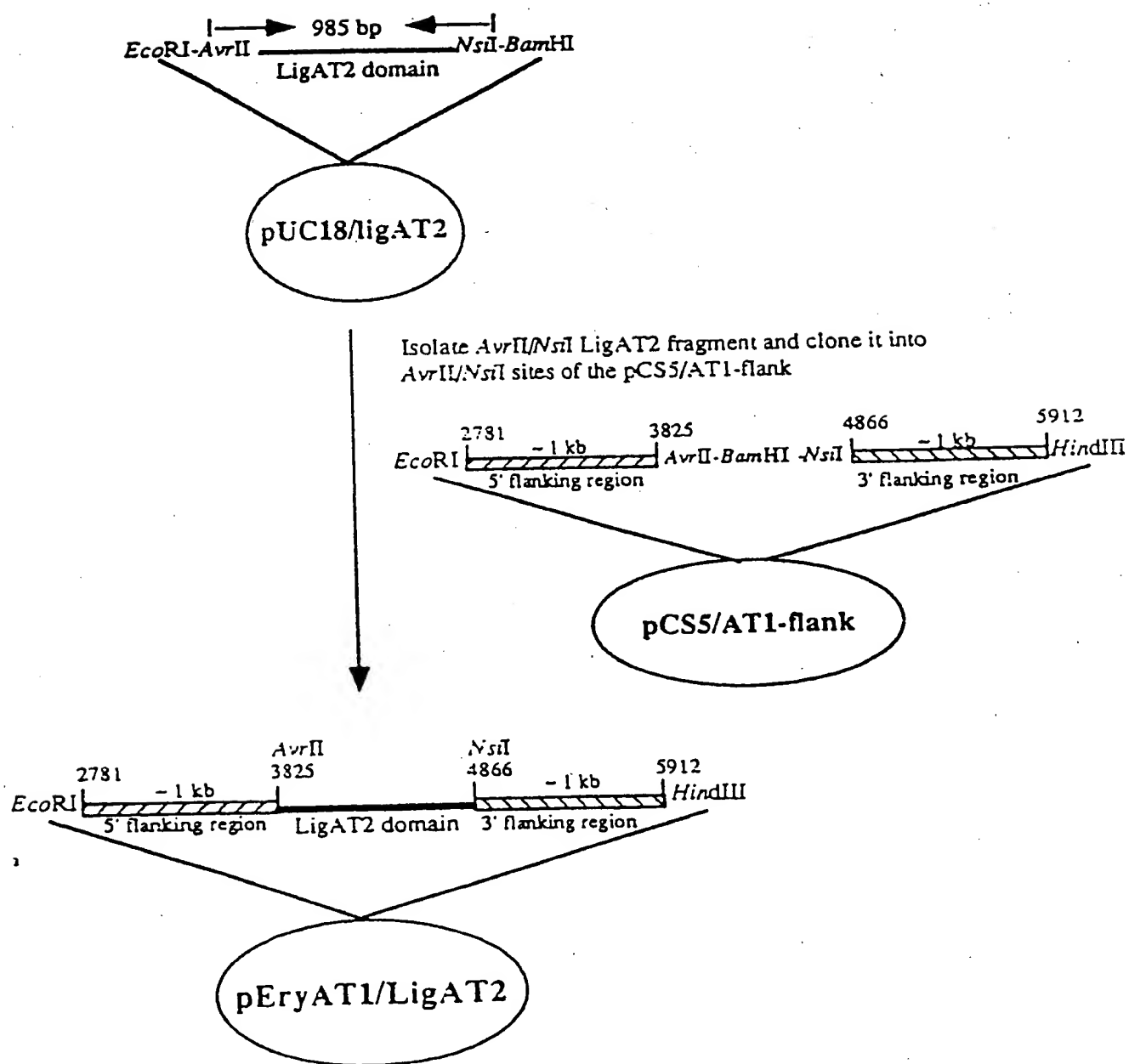
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Figure 9



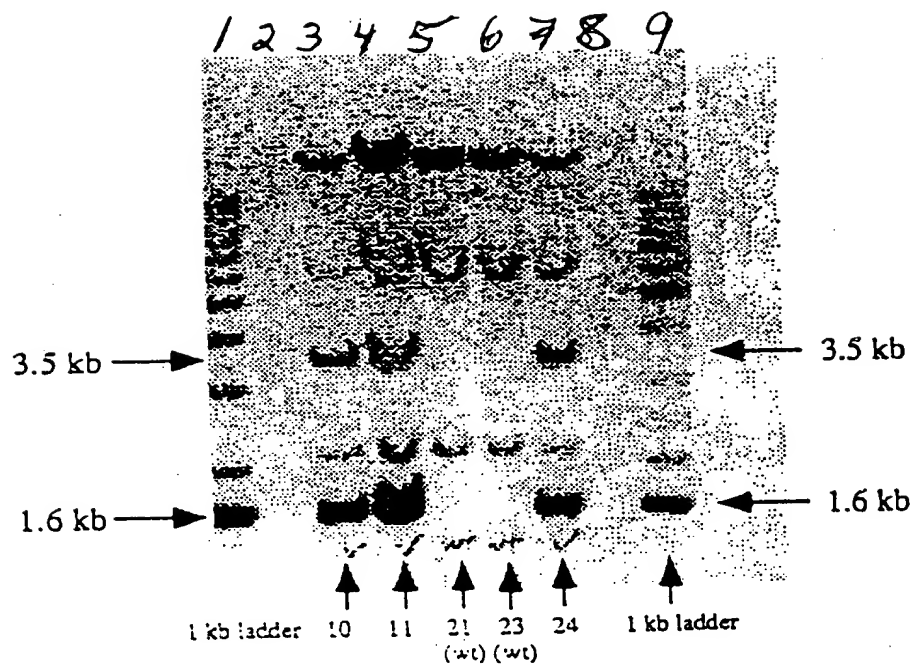
09735056 121100

Figure 10



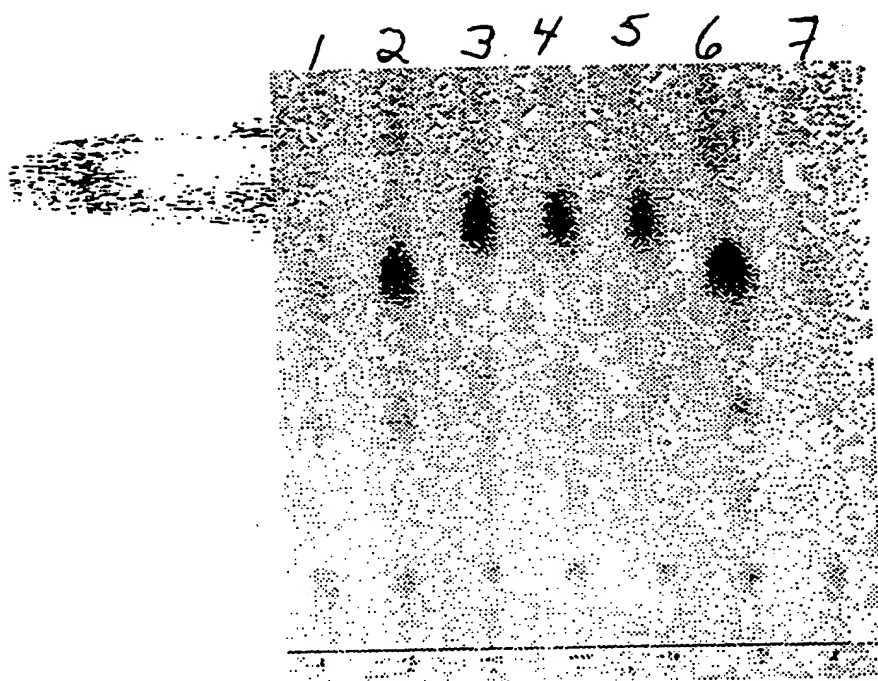
09735056 121100

Figure 11



09735055.121100

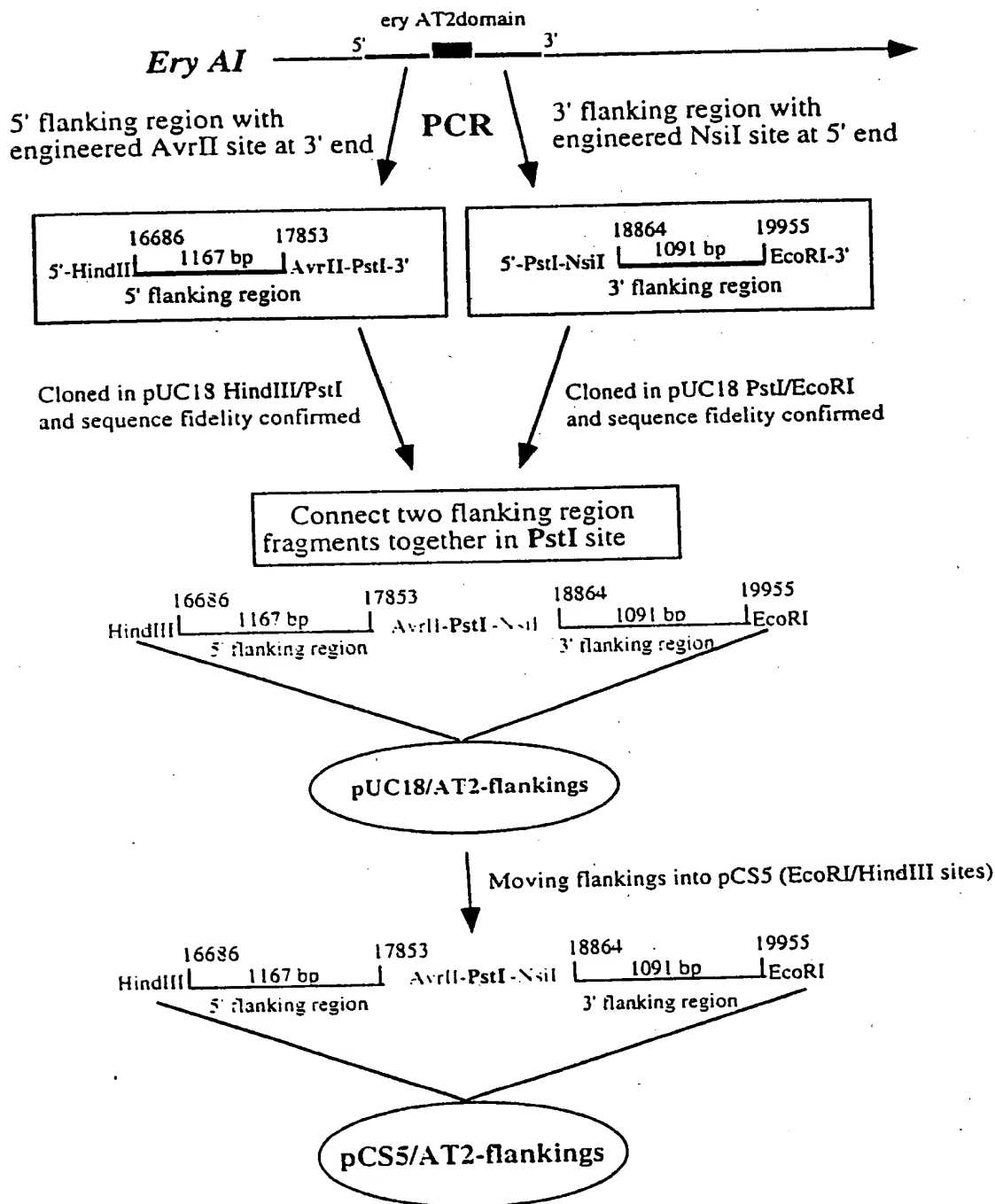
Figure 12



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Figure 13

Construction of eryAT2 flanking regions in pCS5



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Figure 14

Scheme for Construction of pEryAT2/LigAT2 Integration Plasmid

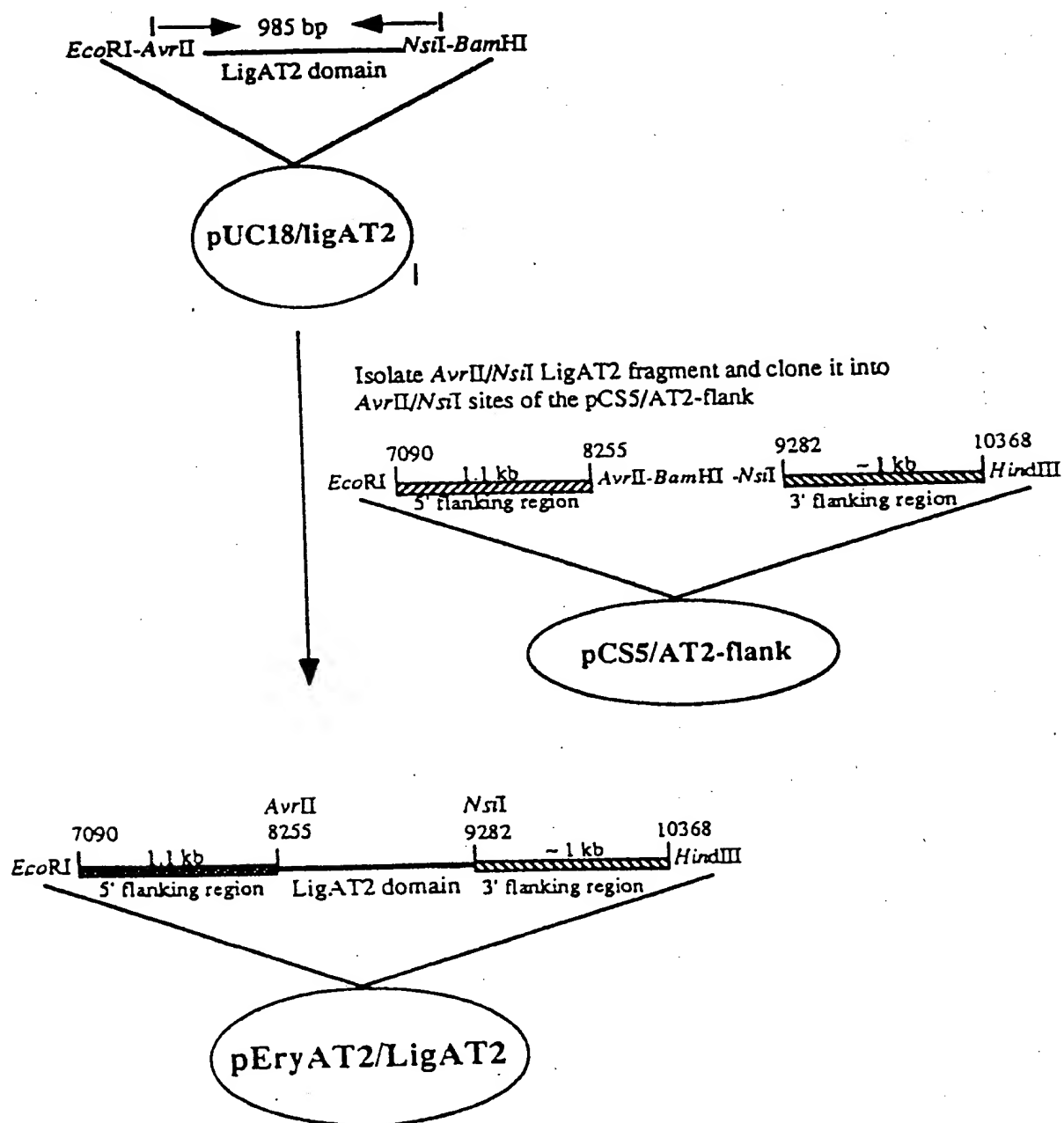
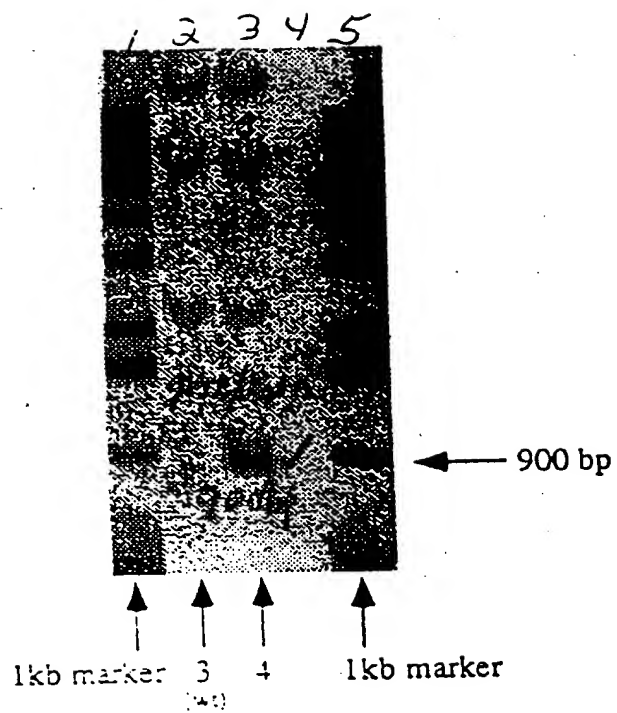
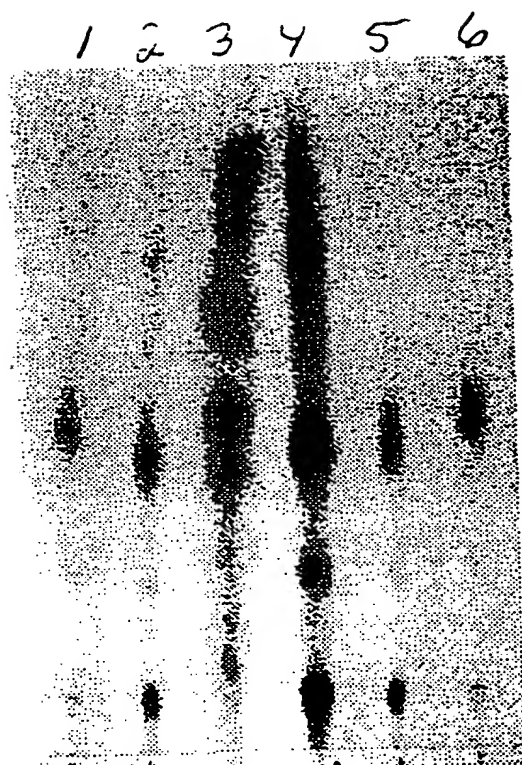


Figure 15



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Figure 16



09735056 121100

Figure 17

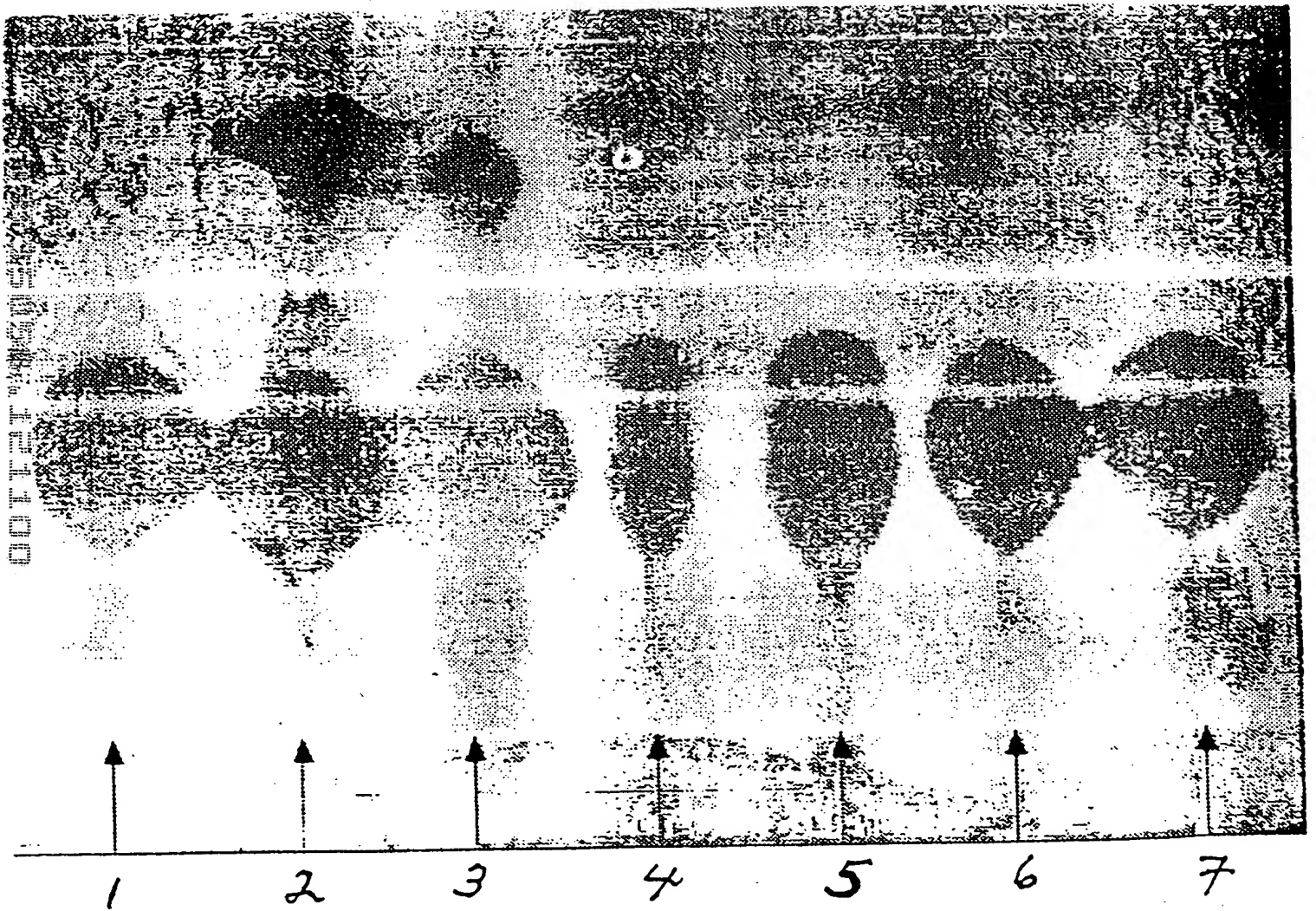


Figure 18

CCTAGGACGGCAGTCCTGCTCACCGGGCAGGGTTCCCAGCGTCAGGGCATGGGGCGCGAA 60
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 CTGTACGACCGGTCACCGGTGTTGCGCCGCTCGTTTCGACGCGATCTGCGCTCAACTCGAC 120
 L Y D R S P V F A A S F D A I C A Q L D 40
 GGGCAACTGCCTCGTCCCCCTCAAGGACGTTCTCTTCGCCCCCGAGGGGTCGGAGGACGCC 180
 G Q L P R P L K D V L F A P E G S E D A 60
 GCGCTCATCGACCGTACGGTGTTCACACAGGCGGGCTCTGTTTCGCGGTGGAGACCTCCCTG 240
 A L I D R T V F T Q A A L F A V E T S L 80
 TTCCGGCTGTTTCGAGGCCCCACGGCCTCGTCCCCGACTACCTCATCGGCCACTCCATCGGC 300
 F R L F E A H G L V P D Y L I G H S I G 100
 GAAGTGACCGCGGCCCCACCTGGCCGGGGTCTCGATCTGGCGGACGCGTGCGTCCTGGTC 360
 E V T A A H L A G V L D L A D A C V L V 120
 GCCCACC GCGGCCGCTGATGCAGTCGGCCCCGGGCGGCGGCGATGGCCGCGGTCCAG 420
 A H R G R L M Q S A R A G G A M A A V Q 140
 GCGAGCGAGGACGAGGTACGCGAGGCCCTCGCGACCTTCGACGATGCGGTTGCCGTGGCC 480
 A S E D E V R E A L A T F D D A V A V A 160
 GGAGTCAACGGCCCCGAACGCCACCGTCGTCTCCGGCGACGAGGACGCGGTTCGAGCGGCTG 540
 G V N G P N A T V V S G D E D A V E R L 180
 GTCGCGCGCTGGCGCGAGCAGGGCAGGCGGACGAAGCGGCTGCCGGTCAGCCACGCCTTC 600
 V A R W R E Q G R R T K R L P V S H A F 200
 CACTCGCCGCACATGGACGGGATCGTCGACGAGTTCGTACCGCCGTCTCCGGGCTCACC 660
 H S P H M D G I V D E F V T A V S G L T 220
 TTCCGCTCCCCGACGATCCCGGTCTCTCCAACGTACCGGGACCCTCGCCACCGTCGAC 720
 F R S P T I P V V S N V T G T L A T V D 240
 CAGCTGACCTCGCCCGCTACTGGGCACGCCACATCCGCGAGGCCGTGCGCTTCGCCGAC 780
 Q L T S P A Y W A R H I R E A V R F A D 260
 GGGGTGCGGTACCTGGAGGGCGAGGGCGTACCGAATGGCTGGAGCTCGGGCCCCGACGGC 840
 G V R Y L E G E G V T E W L E L G P D G 280
 GTTCTCGTCCGCTGGTTCGAGGACTGCCTGGCGAAGGAGGCGGGATCGCTCGCGTCCGCC 900
 V L V A L V E D C L A K E A G S L A S A 300
 CTGCGCAAGGGGGCGAGCGAGCCCCACACCGTGGGCGCGGCCATGGCCCCGCGCGGTGCTG 960
 L R K G A S E P H T V G A A M A R A V L 320
 CGCGGATCCGGCCCCGACTGGGCGGCGGTGTTCCCCGGCGCACGGCGGGTTCGACCTTCGG 1020
 R G S G P D W A A V F P G A R R V D L P 340
 ACGTATGCAT 1030
 T Y A 343

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Figure 19

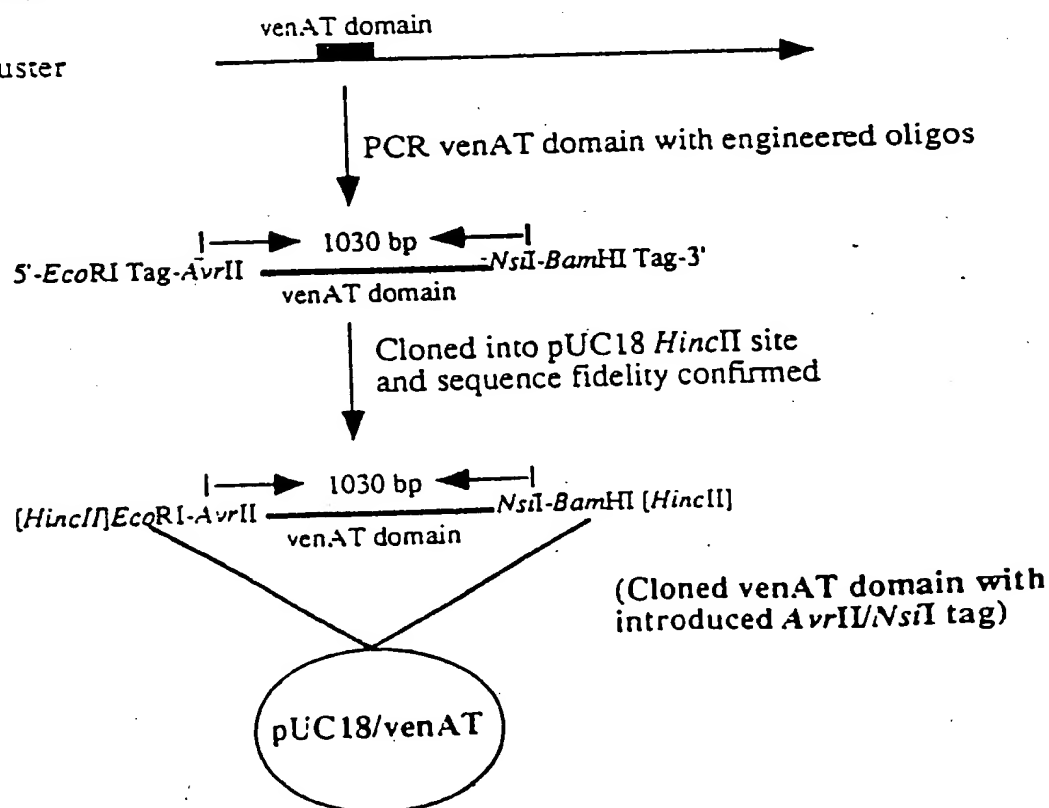
PCR oligos:

N-terminal Oligo: 5' *Eco*RI Tag-^{AvrII}CCTAGGACGGCAGTCCTGCTCACC-3'
GGCC
Engineered *Avr*II | Homologous region

C-terminal Oligo: 5' *Bam*HI Tag-^{NsiI}ATGCATACGTCGGAAGGTCGACCCG-3'
C C
Engineered *Nsi*I | Homologous region

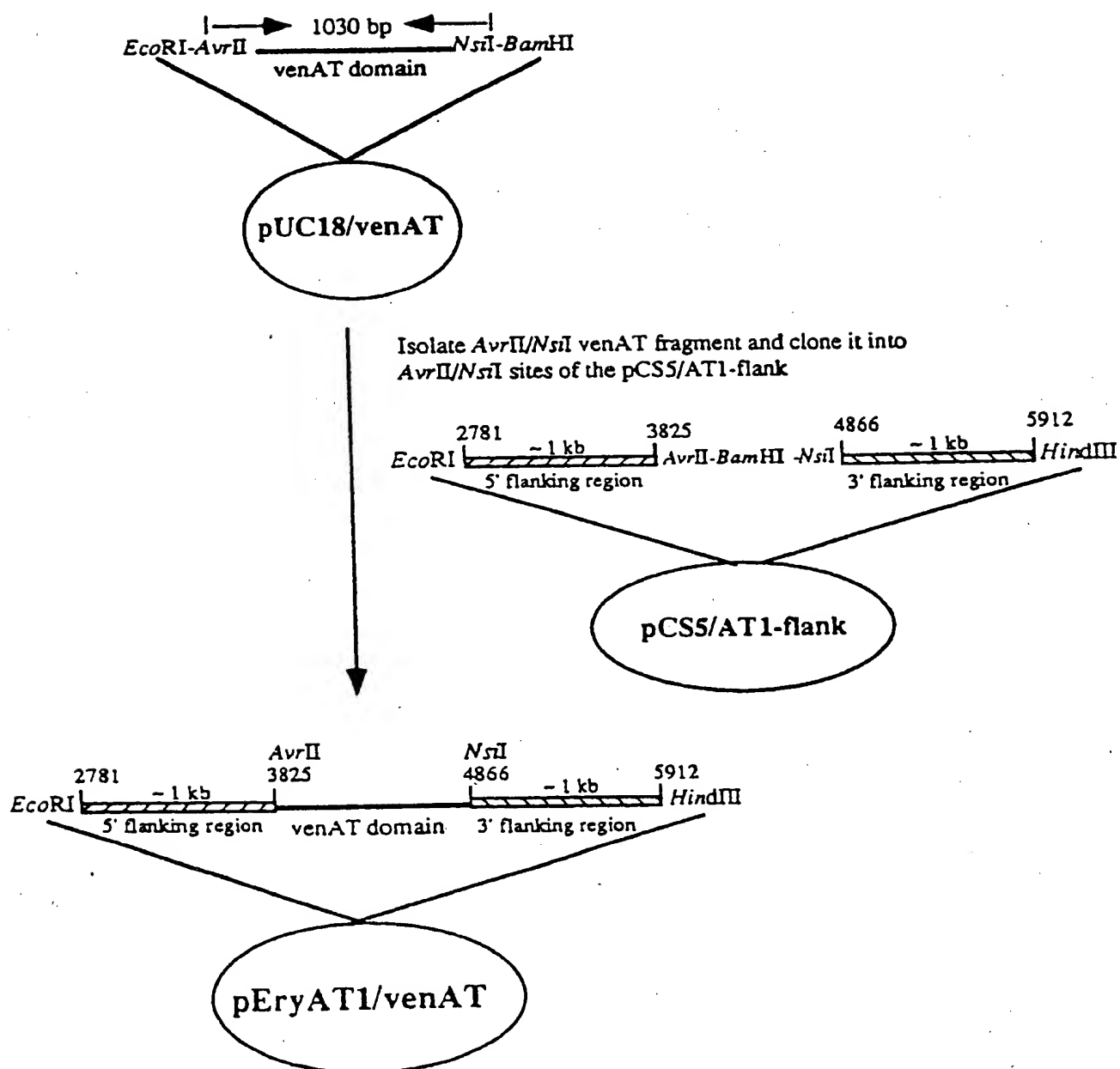
PCR cloning:

Ven-PKS cluster



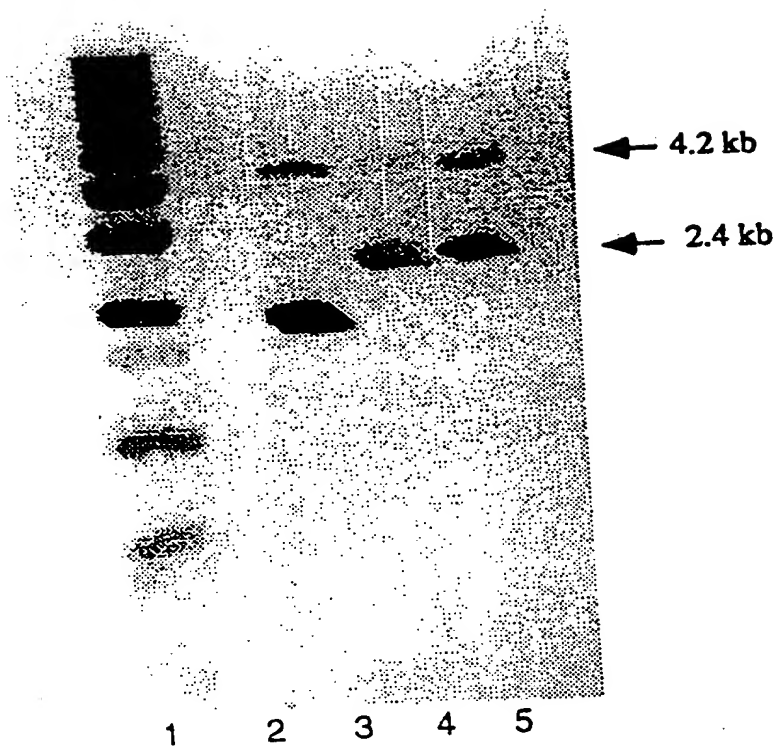
09735056 121100

Figure 20



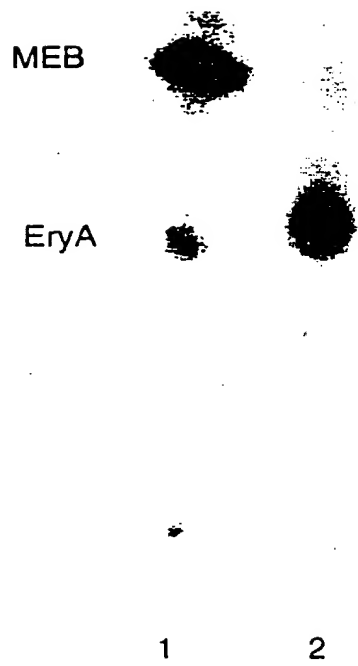
09735056-121100

Figure 21



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Figure 22



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Figure 23

PCR oligos:

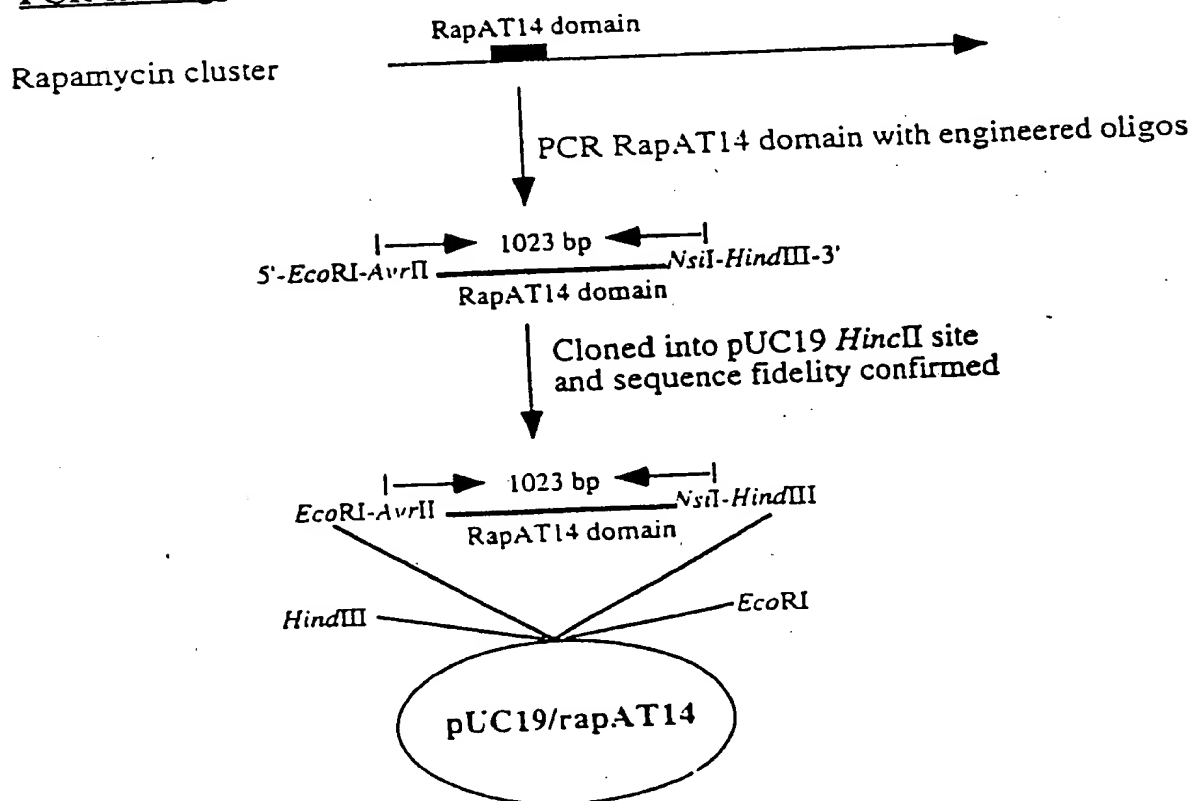
N-terminal Oligo: 5' *EcoRI* Tag- $\overbrace{\text{CCTAGGGTTGCCTTCCTGTTTCGAC}}^{\text{AvrII}}\text{-3'}$
 GGC C

Engineered <i>AvrII</i>	Homologous region
-------------------------	-------------------

C-terminal Oligo: 5' *HindIII* Tag- $\overbrace{\text{ATGCATAGACCGGCAGATCCACCG}}^{\text{NsiI}}\text{-3'}$
 C G

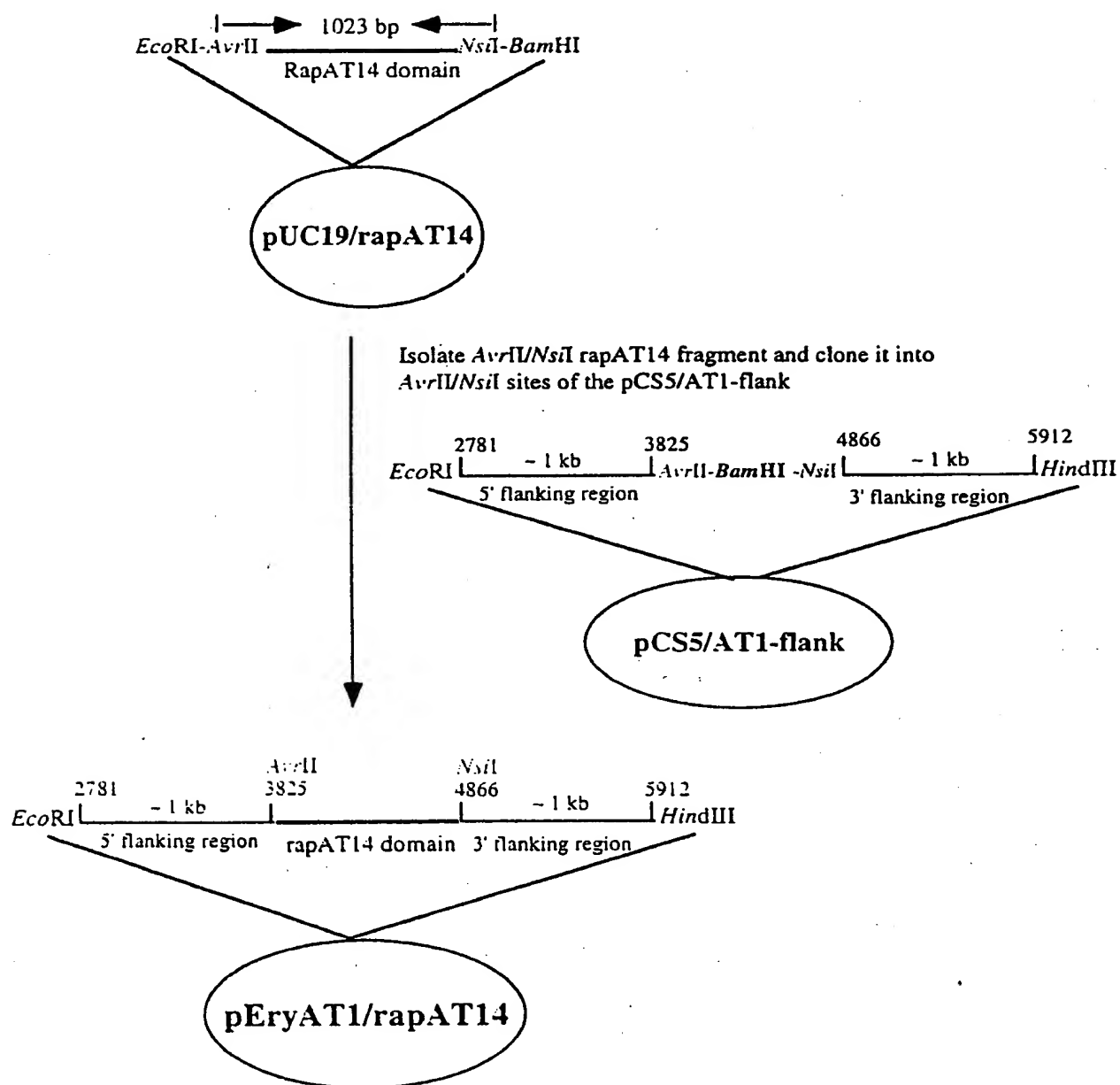
Engineered <i>NsiI</i>	Homologous region
------------------------	-------------------

PCR cloning:



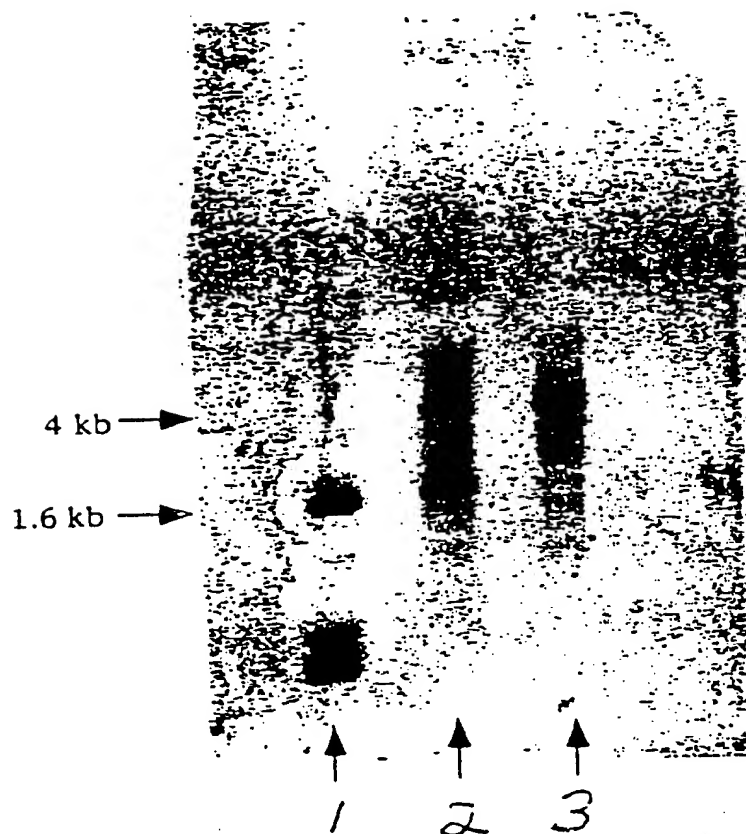
09735056 121100

Figure 24



09735055.121100

Figure 25



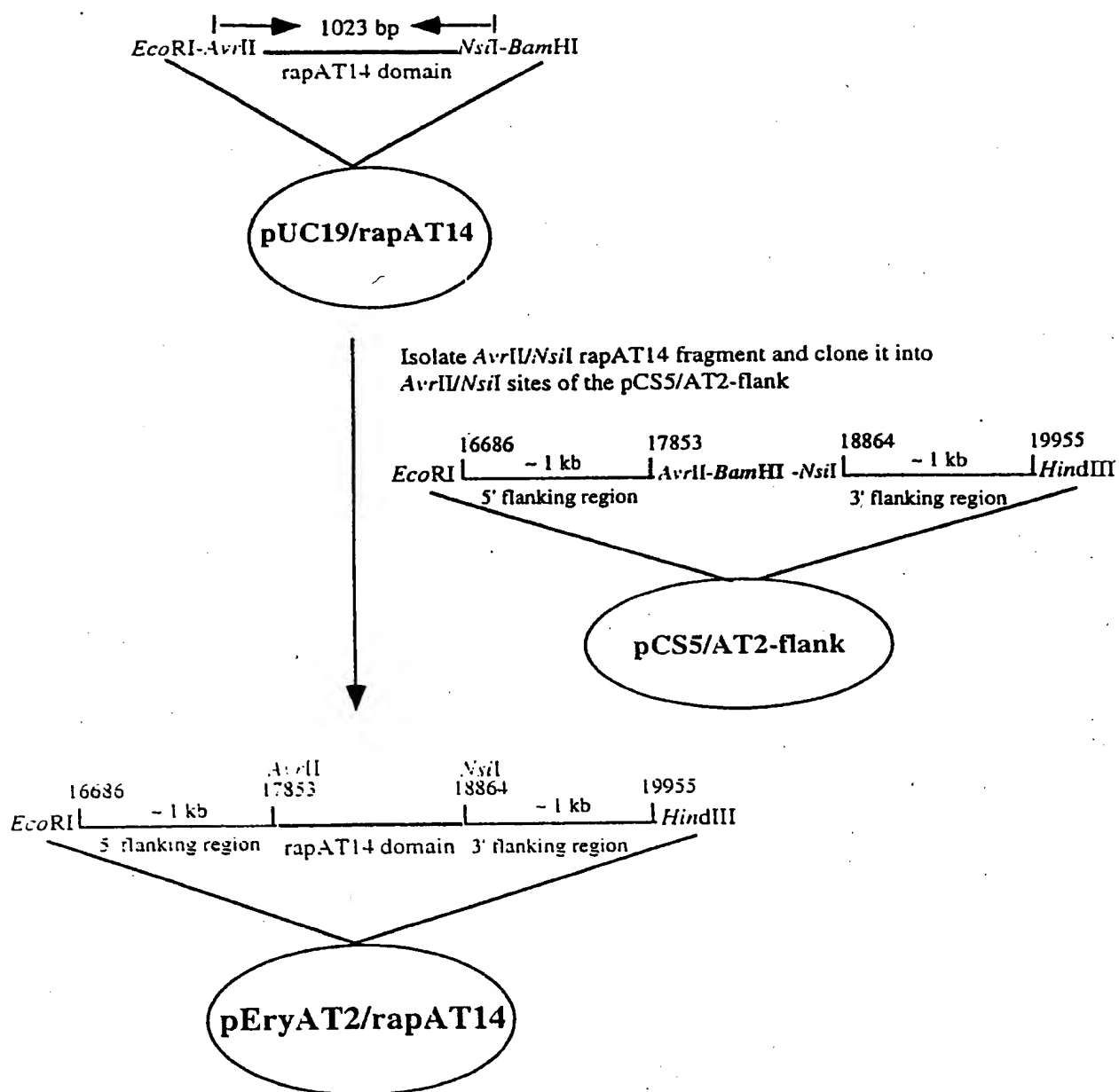
09735056.121100

Figure 26



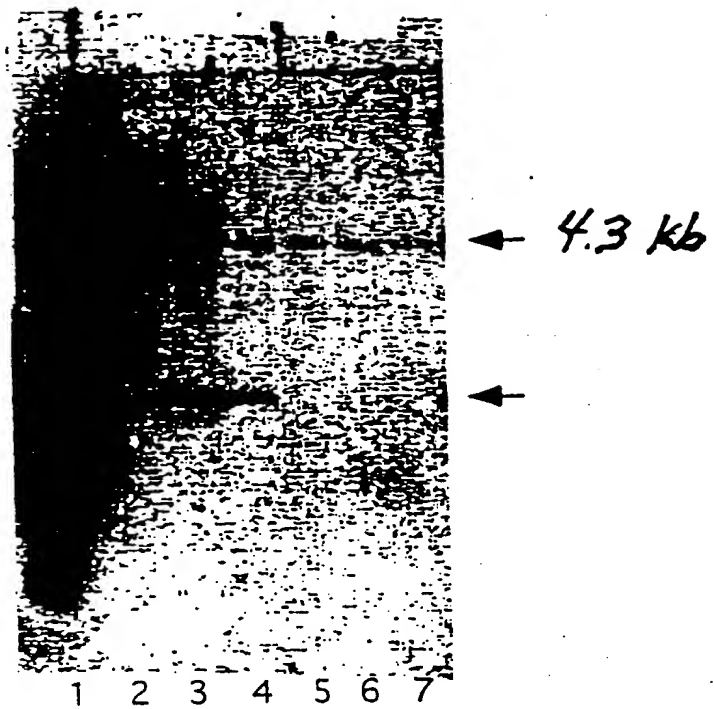
09735056 124100

Figure 27



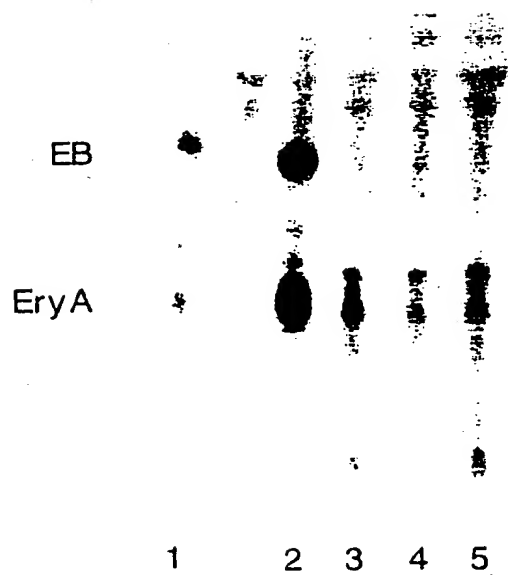
09735056 121100

Figure 28



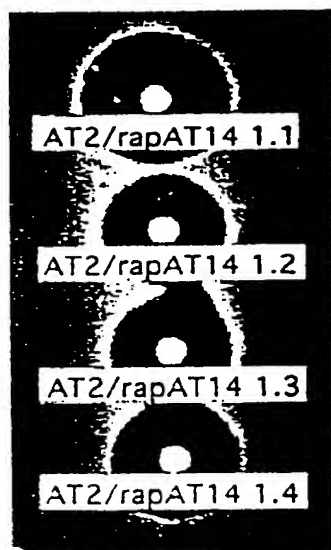
09735056.121100

Figure 29



09735056.121100

Figure 30



09735056 121100

Figure 31

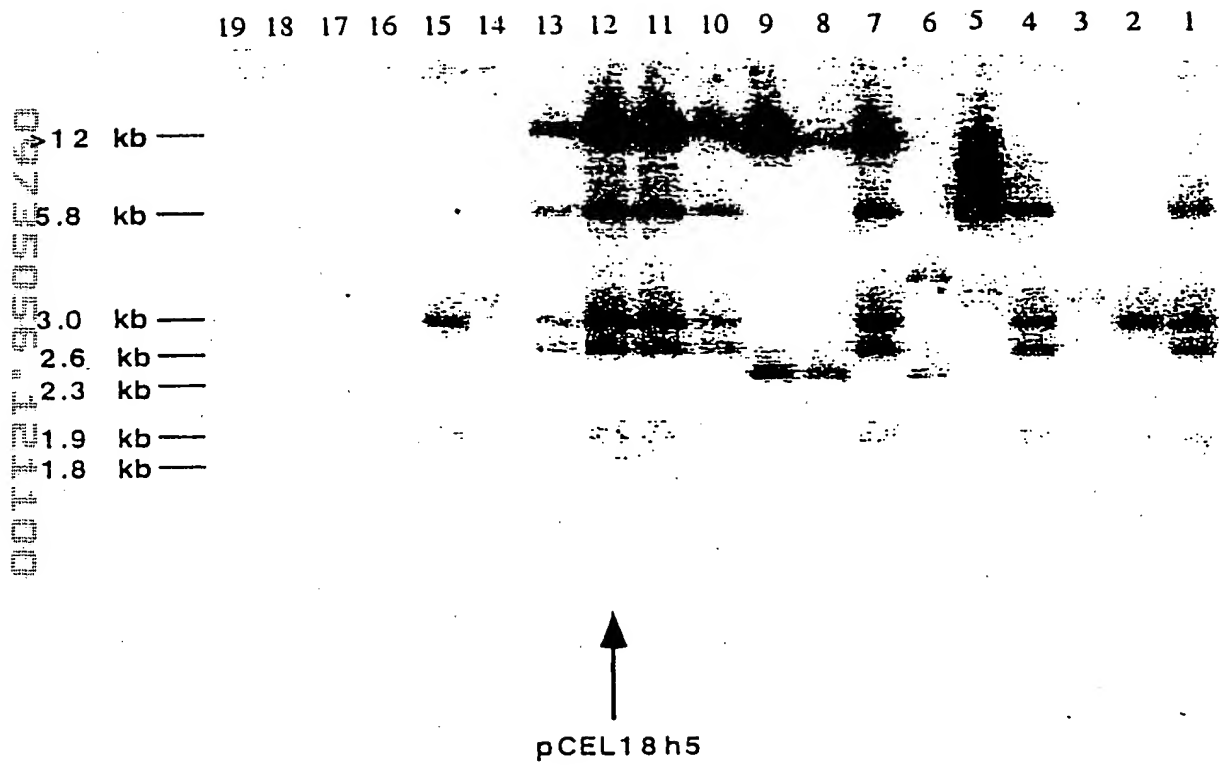


Figure 32

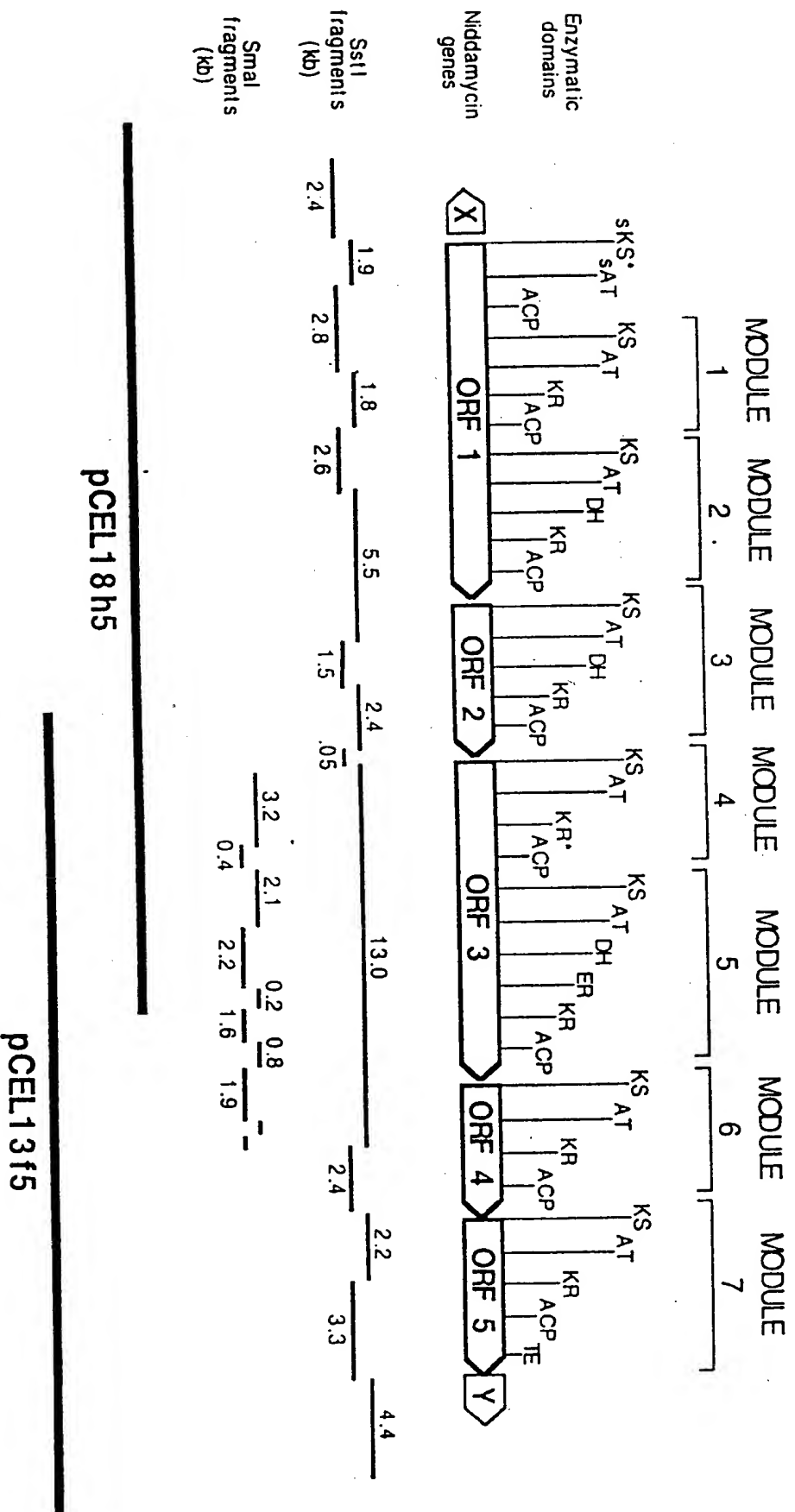
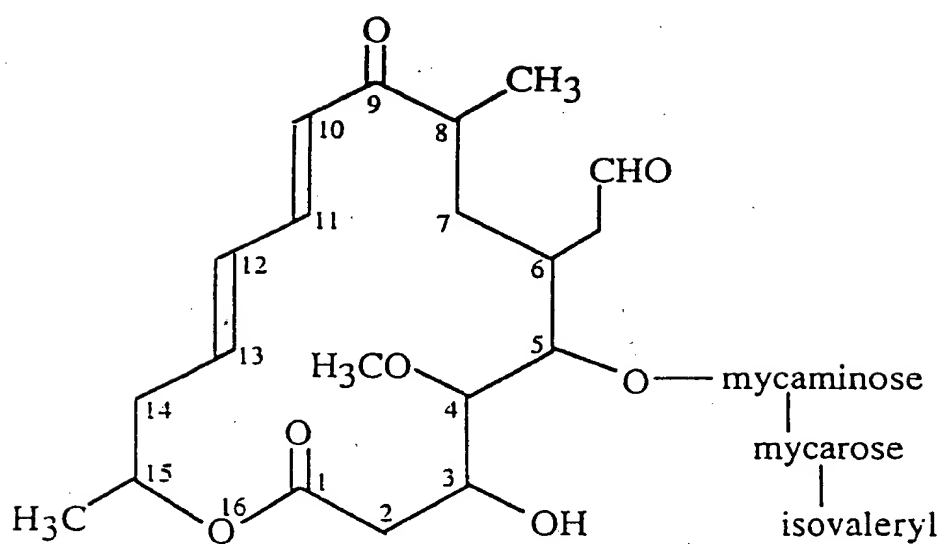


Figure 33



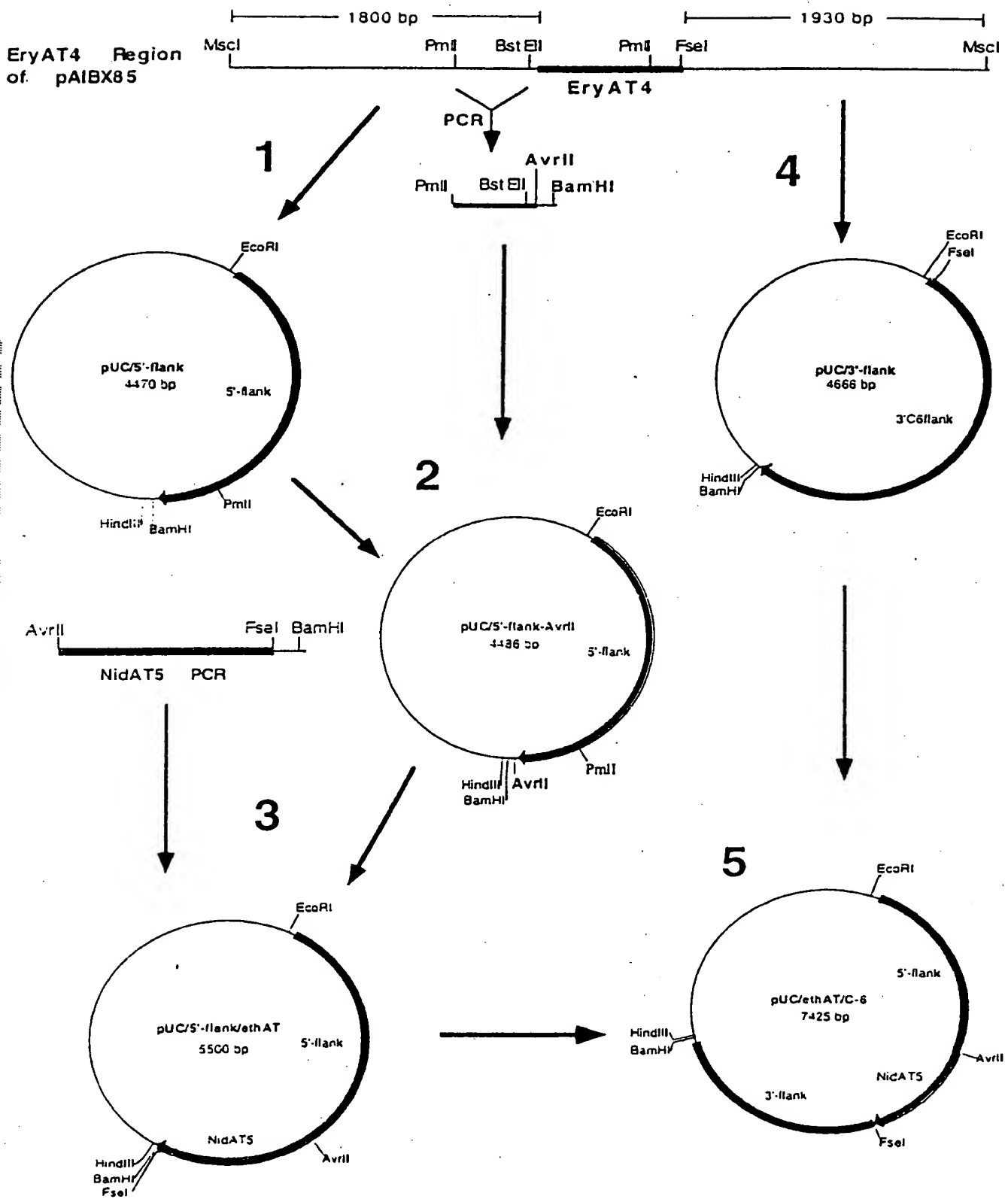
09735056 121100

Figure 34

GCCGACCGTGTCTGTGTTCTGTGTTCCCCGGCCAGGGCTCGCAGTGGGCCCGGAATGGCCGAG 60
 A D R V V F V F P G Q G S Q W A G M A E 20
 GGGCTGCTGGAGCGGTCCGGCGCGTTCGGGAGTGGCGCCGACTCGTGCGACGCCGCGCTG 120
 G L L E R S G A F R S A A D S C D A A L 40
 CGGCCGTACCTCGGCTGGTCGGTGCTGAGCGTGCTGCGCGGGGAACCGGACGCGCCCTCG 180
 R P Y L G W S V L S V L R G E P D A P S 60
 CTCGACCGGGTCGACGTCTGTGCAGCCGGTGCTGTTTACGATGATGGTCTCGCTCGCGGCG 240
 L D R V D V V Q P V L F T M M V S L A A 80
 GTCTGGCGTGCGCTGGGGGTGGAACCGGCGGCGGTCTGTCGGGCACTCGCAGGGTGAGATC 300
 V W R A L G V E P A A V V G H S Q G E I 100
 GCCGCTGCCCATGTCTGCCCGGTGCGCTGTCTGCTGGACGACTCGGCCCGGATCGTCGCCCTG 360
 A A A H V A G A L S L D D S A R I V A L 120
 CGCAGTCGGCGTGCTCGGACTGGCGGGCAAGGGCGGCATGGTGGCGGTGCCGATGCCG 420
 R S R A W L G L A G K G G M V A V P M P 140
 GCGGAGGAGCTGCGGCCCGGGGTGGTGACGTGGGGGGACCGTCTGGCCGTGCGCCGCCGTC 480
 A E E L R P R L V T W G D R L A V A A V 160
 AACAGCCCCCGTTTCTGCGCCGTGCGCAGGCGACCCGGAGGCGCTGGCCGAACCTGGTGGCG 540
 N S P G S C A V A G D P E A L A E L V A 180
 CTGCTGACCGGTGAGGGGGTGACGCCCCGGCCGATCCCCGGCGTCTGACACGGCGGGCCAC 600
 L L T G E G V H A R P I P G V D T A G H 200
 TCGCCCGAGGTGGAGCGCTTGGCGGGCTCATCTGCTGGAGGTGCTGGCCCCGGTGGCCCCC 660
 S P Q V D A L R A H L L E V L A P V A P 220
 CGACCGGGCGACATCCCGTTCTACTCGACGGTGACCGGGCGGGCTGCTGGACGGCACCGAG 720
 R P A D I P F Y S T V T G G L L D G T E 240
 CTGGACGCGACGTAAGTACCGCAACATGCGCGAGCCCGTCTGAGTTCGAGCGGGCCACA 780
 L D A T Y W Y R N M R E P V E F E R A T 260
 CGGGCGCTGATCGCCGACGGGCACGACGTCTTCTGAGAGACGAGCCCGCATCCCATGCTG 840
 R A L I A D G H D V F L E T S P H P M L 280
 GCCGTGGCGCTGGAGCAGACGGTCACCGACGCGCGGCACCGACGCGGGCGGTGCTCGGGACC 900
 A V A L E Q T V T D A G T D A A V L G T 300
 CTGCCCCCGCCGACGGCGGTCTCTGCGCGCTGGCCCTGGCCGTCTGCGCGCCTTCGCG 960
 L R R R H G G P R A L A L A V C R A F A 320
 CACGGCGTGGAGGTGGAACCCCGAGGCGGTCTTCGGTCCGGCGCACGGCCCGTGGAGTTG 1020
 H G V E V D P E A V F G P G A R P V E L 340
 CCCACCTATCCG 1032
 P T Y P 344

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Figure 35



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Figure 36

Protein Sequence	S A P R K P
Original Sequence	TCCGCGCCGCGCAAGCCG
Altered Sequence	TCCGCGCCTAGGAAGCCG

PCR Oligos for 5'-flank AvrII site

N-Terminal oligo (Seq 10 no. 21) 5'-GAGAGAGGAACCAACGCGCACGTGATCGTCGAAGAGGCACCAGC

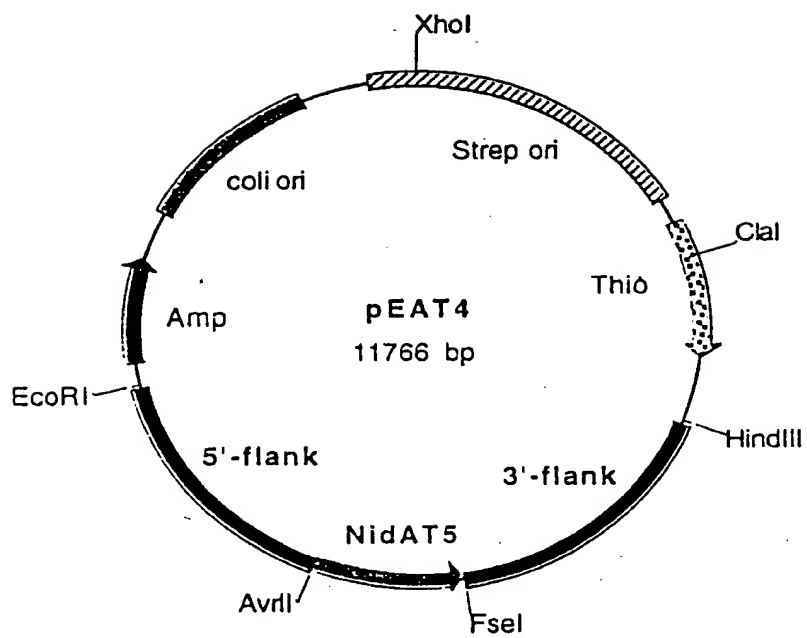
C-terminal oligo (Seq 10 no. 22) 5'-GAGAGAGGATCCGACCTAGGCGCGGAGGTCACCGGCGCGACGGCG

PCR oligos for NidAT5 fragment

N-Terminal oligo (Seq 10 no. 23) 5'-GAGAGACCTAGGAAGCCGGTGTTCGTGTTCCCCGGCCAGGGCT

C-terminal oligo (Seq 10 no. 24) 5'-GAGAGAGGATCCGAGGCCGGCCGTGCGCCCGGACCGAAGACCGCCTC

Figure 37



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Figure 38

001121 9505E260

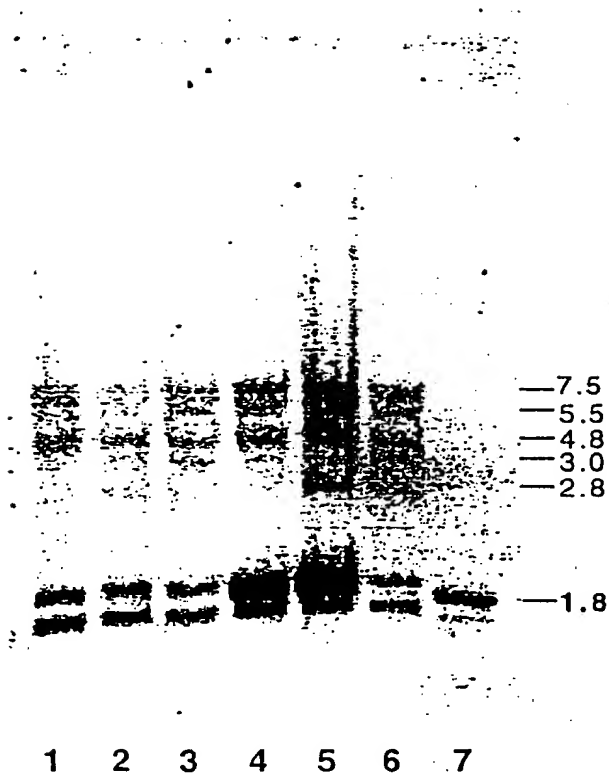
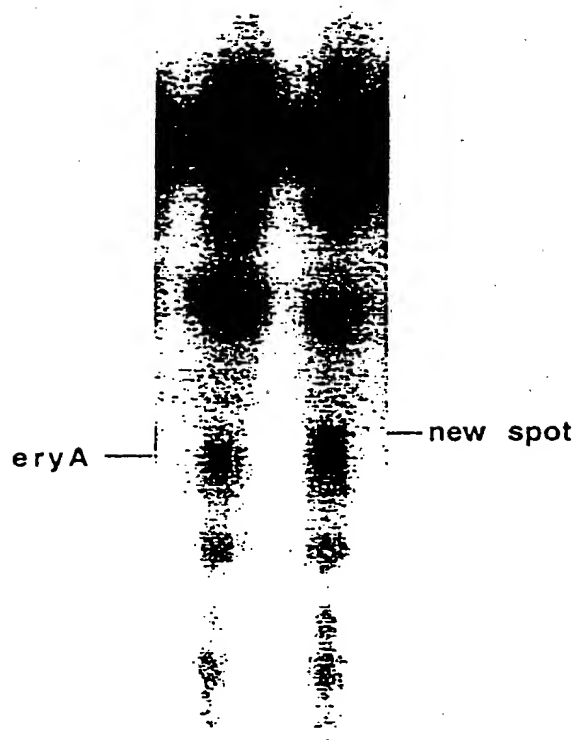


Figure 39

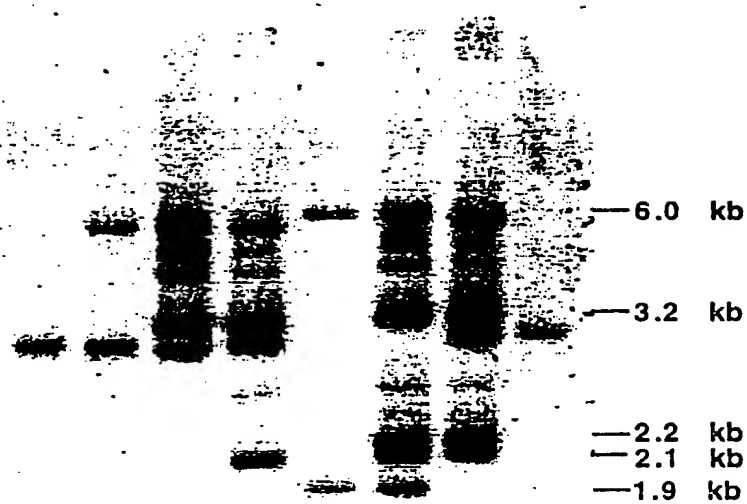


A) SCM only

B) SCM + 50mM butyric acid

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Figure 40



↑
pCEL18h5

↑
pCEL13f5

09735056.121100

Figure 41

CGCGCGCCTGCCTTCGTCTTTCCCGGGCAGGGCGCCAGTGGGCCGGACTGGGAGCGCGG 60
R A P A F V F P G Q G A Q W A G L G A R 20

CTCCTCGCGGACTCCCCGTCTTCCCGGCCAGGGCCGAGGCATGCGCGCGGGCGCTGGAG 120
L L A D S P V F R A R A E A C A R A L E 40

CCTCACCTCGACTGGTTCGGTCTCGACGTGCTGGCCGGCGCCCCGGGCACCCCTCCCATC 180
P H L D W S V L D V L A G A P G T P P I 60

GACCGGGCCGACGTGGTGCAGCCGGTGTGTTCACCACGATGGTCTCGCTGGCCGCCCTC 240
D R A D V V Q P V L F T T M V S L A A L 80

TGGGAGGCCCCACGGGGTGGCGCCGGCCGCGGTCTGGGCCACTCCCAGGGCGAGGTGGCC 300
W E A H G V R P A A V V G H S Q G E V A 100

GCGGCCTGCGTGGCCGGTGCCCTGTGCTGGACGACGCTGCCCTGGTGATCGCCGGACGC 360
A A C V A G A L S L D D A A L V I A G R 120

AGCAGGCTGTGGGGGCGGCTGGCCGGGAACGGCGGGATGCTCGCGGTGATGGCTCCGGCC 420
S R L W G R L A G N G G M L A V M A P A 140

GAGCGGATCCGTGAGCTGCTCGAACCATGGCGGCAGCGGATTTTCGGTGGCGGCGGTCAAT 480
E R I R E L L E P W R Q R I S V A A V N 160

GGCCCCGCTCGGTACCGTCTCCGGTGACGCGCTCGCGCTGGAGGAGTTCGGCGCGCGG 540
G P A S V T V S G D A L A L E E F G A R 180

CTCTCCGCGGAGGGGGTGTGCGCTGGCCGCTGCCGGGCGTTCGACTTCGCCGGCCACTCG 600
L S A E G V L R W P L P G V D F A G H S 200

CCGCAGGTGGAGGAGTTCGCGCTGAGCTCCTGGACCTGCTCTCCGGCGTACGGCCGGCT 660
P Q V E E F R A E L L D L L S G V R P A 220

CCTTCGCGGATACCTTTCTTCTCCACCGTGACGCGGGTCTTTCGCGCGGCGACCAGCTG 720
P S R I P F F S T V T A G P C G G D Q L 240

GACGGGGCGTACTGGTACCGCAACACGCGGAACCCGTGGAGTTCGACGCCACGGTCCGG 780
D G A Y W Y R N T R E P V E F D A T V R 260

GCGCTGCTGCGTGCGGGCCATCACACGTTCATCGAGGTGGTCCGATCCGCTGCTCAAC 840
A L L R A G H H T F I E V G P H P L L N 280

GCCGCGATCGACGAGATCGCAGCGGACGAGGGGGTAGCGGCCACGGCCCTGCATACGCTC 900
A A I D E I A A D E G V A A T A L H T L 300

CAGCGGGGCGCTGGCGGCCTTGACCGCGTGCGCAACGCGGTGGGCGCCGCTTTCGCGCAC 960
Q R G A G G L D R V R N A V G A A F A H 320

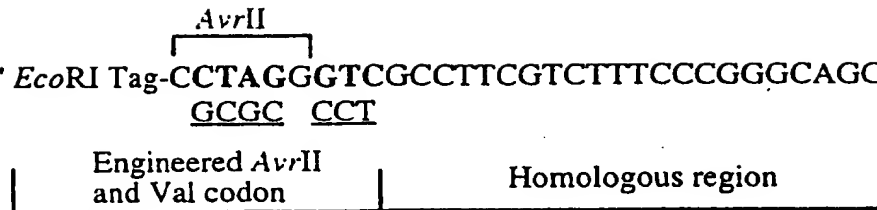
GGTGTCCGGGTCGACTGGAACGCCCTGTTCGAGGGCACCGGTGCGCGCAGGGTGCCGCTT 1020
G V R V D W N A L F E G T G A R R V P L 340

CCCTCGTACGCCTTC 1035
P S Y A F 345

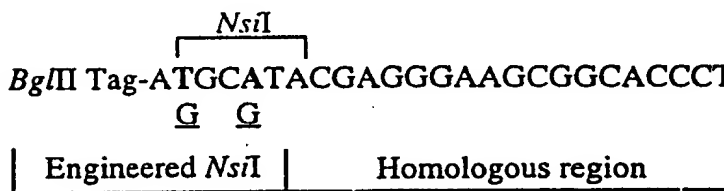
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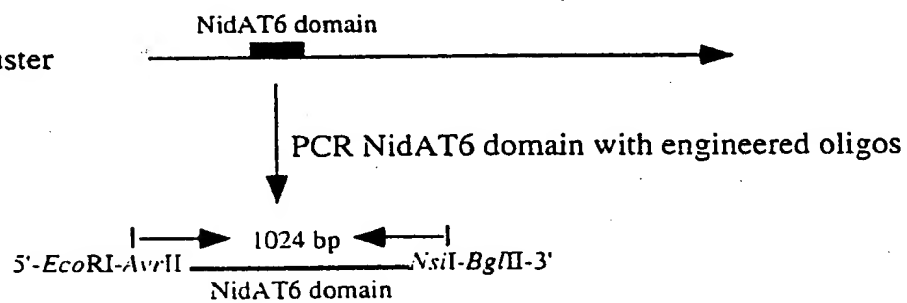
N-terminal Oligo: 5' *Eco*RI Tag-CCTAGG^{AvrII}GTCGCCTTCGTCTTTCCCGGGCAGG-3'
GCGC CCT



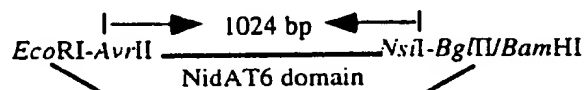
C-terminal Oligo: 5' *Bgl*III Tag-ATGCATACGAGGGAAGCGGCACCCTGC-3'



The diagram illustrates the Nidamycin cluster and the NidAT6 domain. A horizontal line represents the Nidamycin cluster, with a black box indicating the NidAT6 domain. An arrow points to the right from the NidAT6 domain. Below the NidAT6 domain, a vertical line connects to the text "PCR NidAT6 domain with engineered oligos".



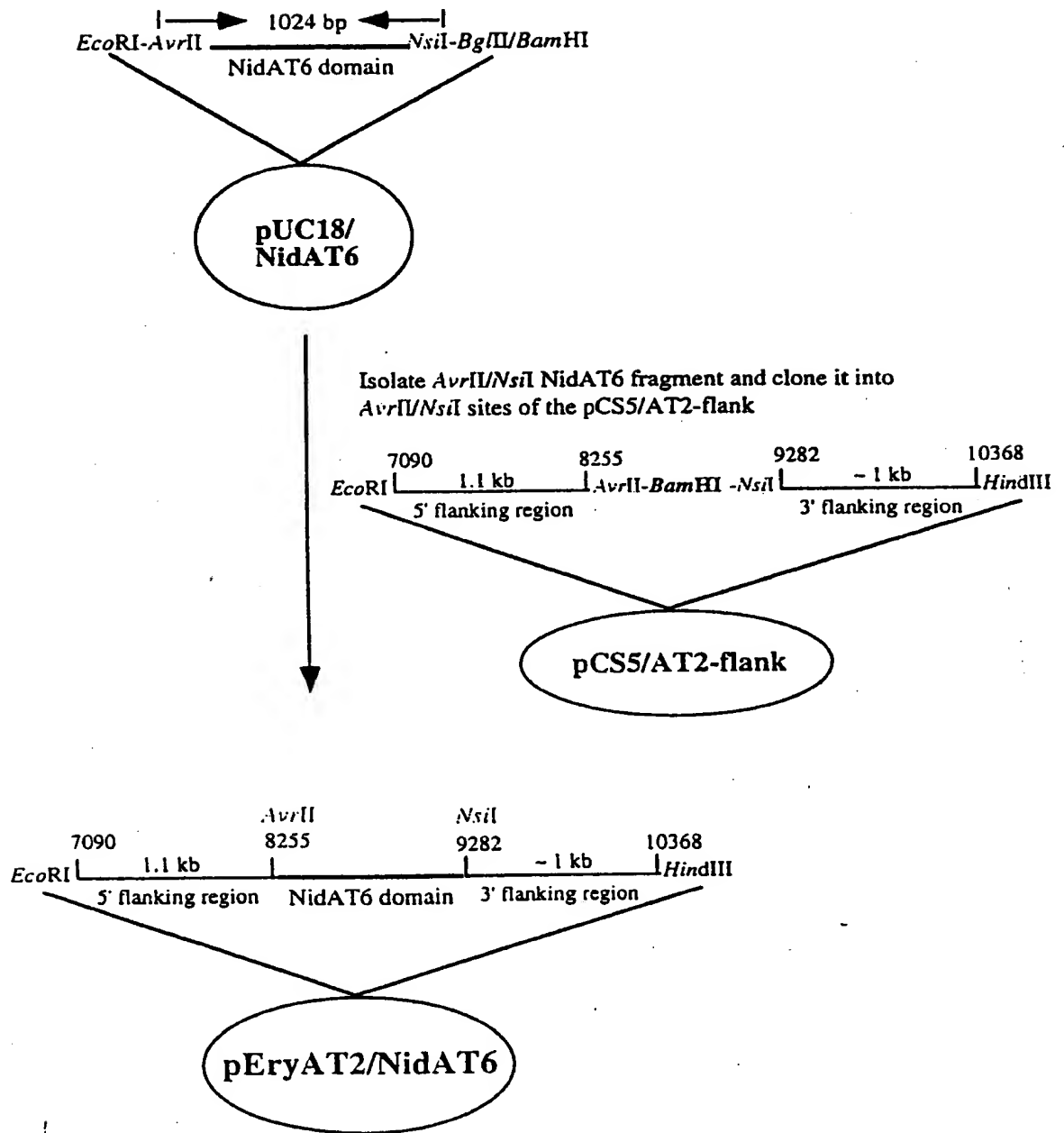
Cloned into pUC18 *EcoRI*-*Bam*HI sites
and sequence fidelity confirmed



(Cloned NidAT6 domain with introduced *AvrII*/*NsiI* sites)



Figure 43



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